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<p>(71) Applicant (for all designated States except US): TRUSTEES OF THE UNIVERSITY OF PENNSYLVANIA [US/US]; Suite 300, 3700 Market Street, Philadelphia, PA 19104-3147 (US).</p> <p>(72) Inventor; and (75) Inventor/Applicant (for US only): EL-DEIRY, Wafik, S. [US/US]; Apartment P113, 1500 Locust Street, Philadelphia, PA 19102 (US).</p> <p>(74) Agents: REED, Janet, E. et al.; Dann, Dorfman, Herrell and Skillman, Suite 720, 1601 Market Street, Philadelphia, PA 19103 (US).</p>																					

(54) Title: NUCLEIC ACID ENCODING A NOVEL CHEMOTHERAPY-INDUCED PROTEIN, AND METHODS OF USE

(57) Abstract

An isolated nucleic acid molecule is provided which encodes a mammalian signal transducing protein (Killer) involved in the induction of apoptosis. The encoded protein comprises an amino-terminal extracellular domain, a hydrophobic transmembrane domain, and a carboxy-terminal death domain that can form protein-protein interactions with other death domain-containing proteins to transmit the signals required for apoptosis. The invention also provides the Killer protein and antibodies thereto. These biological molecules are useful as diagnostic and therapeutic agents for the identification, detection and regulation of complex signaling events leading to apoptotic death. The molecules may also be used to advantage for assessing the potential chemosensitivity of a targeted tumor cell. Additionally, these molecules may be used as research tools to facilitate the elucidation of the mechanistic action of the Killer proteins of the invention.

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**NUCLEIC ACID ENCODING A NOVEL CHEMOTHERAPY-INDUCED
PROTEIN, AND METHODS OF USE**

This application claims priority to the following United States Provisional Applications, all of which are incorporated by reference herein: 60/052,305, filed July 11, 1997; 60/054,710, filed August 4, 1997; 5 60/060,473, filed September 30, 1997; 60/077,661, filed March 11, 1998; 60/077,628, filed March 11, 1998; and 60/077,526, filed March 11, 1998.

FIELD OF THE INVENTION

10 This invention relates to diagnosis and treatment of neoplastic diseases. More specifically, this invention provides novel nucleic acid molecules, proteins and antibodies useful for detection and/or regulation of neoplastic cellular changes.

15

BACKGROUND OF THE INVENTION

Several publications are referenced in this application to more fully describe the state of the art to which this invention pertains. The disclosure of each 20 such publication is incorporated by reference herein.

The p53 tumor suppressor protein is a nuclear phosphoprotein that functions in cell-cycle arrest, programmed cell death (apoptosis), inhibition of tumor growth, and preservation of genetic stability. It 25 performs these functions through involvement in several biochemical pathways, including transcriptional activation, transcriptional suppression and inhibition of DNA replication. Loss of proper p53 function in cells is one step in the progression toward a neoplastic phenotype; more than 50% of human cancers have mutations in the p53 gene (see V.E. Velculescu & W.S. El-Deiry, Clin. Chem., 42:858-868, 1996; A.J. Levine, Cell 30 88: 321-331, 1997).

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DNA binding and transcriptional activation are among the best-understood functions of p53. Nearly every tumor-derived p53 mutant has lost its ability to bind DNA and transcriptionally activate nearby genes. This 5 observation suggests that these properties of p53 are critical to its role in the control of cell proliferation. The diverse nature of the genes that p53 transcriptionally regulates suggests that p53 may be involved in pathways of cell-cycle control, angiogenesis, 10 DNA repair, differentiation, growth factor signaling and apoptosis.

Experimental evidence indicates that p53 activates a cell suicide pathway (apoptosis) after treatment with chemo- or radiotherapy. In some cell 15 types, the cytotoxic action of anti-cancer agents may be mediated through p53-dependent apoptosis. p53 regulates a number of genes involved in apoptosis, including *bax*, *bcl-2*, insulin-like growth-factor binding protein-3 (*igf-bp3*), and *fas*. The genes for *Bax* and *IGF-BP3* have been 20 shown to contain p53-dependent, *cis*-acting, DNA-responsive elements.

Inactivation or mutation of p53 has been reported to enhance cell sensitivity to cytotoxic agents which induce DNA damage. However, the status (wild type 25 or mutant) of p53 in tumor cells does not always correlate with chemosensitivity. Endogenous p53 status predicts *in vitro* chemosensitivity to the clinically useful agents Adriamycin, Carboplatinum, Cytoxan, and Etoposide in only a minority of cancer cell lines 30 (ovarian and some Burkitt's lymphoma cells), but not in others (e.g., leukemia or lung cancer cells). Several recent studies have shown that many clinically useful drugs appear to be more effective in *in vitro* killing of tumor cell lines that contain wild-type p53. 35 Furthermore, HPV16 E6-mediated degradation of p53 in a human lung cancer cell line does not render these cells more resistant to Adriamycin or Etoposide. By contrast,

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human ovarian teratocarcinoma cells become more chemoresistant and display less death by apoptosis when the endogenous p53 protein is degraded by E6 expression. Additionally, mutations in the p53 gene do not correlate 5 with radioresistance in a series of human squamous carcinoma cells. Finally, recent studies have shown decreased patient survival in patients who have aggressive tumors which have lost p53 following chemo- or radiotherapy (Velculescu, et. al., 1996, *supra*).

10 Certain cytokines interacting with their cognate receptors on the surface of target cells also trigger apoptosis. For example, Fas ligand (FasL) and tumor necrosis factor (TNF) are members of the TNF family of cytokines that trigger apoptosis in target cells 15 through binding and trimerization of their respective cell surface receptors, Fas and TNF receptor 1 (TNFR1). Another TNF family cytokine is TRAIL, the cytotoxic ligand for the recently cloned DR4 receptor (Pan et al., Science 276: 111-113, 1997). Once trimerized, the death 20 domains in the C-termini of the receptors form protein-protein interactions with the C-terminal death domains of intracellular death signal effector molecules, initiating a cascade of signals (involving activation of caspases and endonucleases) that ultimately cause apoptosis.

25 Wild-type p53 has been shown to upregulate production of Fas/APO-1 mRNA and protein, but as yet, no p53-responsive DNA binding site has been identified in the *fas* gene. Thus p53 has not been shown to directly regulate the transcription of a TNFR family member in an 30 apoptosis pathway.

It is clear from the foregoing discussion that the relationship between p53 status and chemo- and radiosensitivity is not sufficiently understood to warrant its use in cancer therapy. As set forth above, 35 p53 status in a tumor does not necessarily predict therapeutic outcome following such treatment. Moreover, gene replacement therapy with wild-type p53 may be

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ineffective, and for some tumors counterproductive, as it is not as yet predictable which tumors would respond positively or negatively to such therapy. For these reasons, there is a need to identify downstream targets 5 of p53 function in apoptotic signaling. Once identified, such p53 targets may be utilized or regulated in apoptosis-based therapeutic approaches to the elimination of tumor cells.

10 **SUMMARY OF THE INVENTION**

The present invention provides novel nucleic acid molecules that are themselves targets of p53 activity and whose encoded proteins are involved in modulation of cellular proliferation. According to one 15 aspect of the present invention, an isolated nucleic acid molecule is provided that includes an open reading frame encoding a mammalian p53-inducible, apoptosis-mediating protein. The protein comprises an amino-terminal extracellular domain, a transmembrane domain, and a death 20 domain that facilitates protein-protein interactions.

In a preferred embodiment of the invention, the nucleic acid molecule is of human origin, and comprises a sequence substantially the same as SEQ ID NO:1, or at least substantially the same in the death domain-encoding 25 region as SEQ ID NO:1. The invention also comprises a nucleic acid having the sequence of SEQ ID NO:1. The encoded protein, which is referred to herein as "Killer," for reasons described below, preferably comprises a sequence substantially the same as SEQ ID NO:2, or at 30 least substantially the same in the death domain of SEQ ID NO:2. A Killer protein encoded by SEQ ID NO:2 is another preferred embodiment of the invention.

In another aspect of the invention, oligonucleotides that specifically hybridize with 35 selected portions of the nucleic acid molecules of the invention are provided.

In yet another aspect of the invention,

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antibodies immunologically specific for the Killer proteins of the invention, or fragments thereof, are provided.

According to other aspects of the present invention, uses of the nucleic acid molecules of the invention, their encoded proteins, as well as antibodies to those proteins, for diagnostic and therapeutic purposes are provided. Specifically, methods are provided for assessing the induction of "Killer" expression following exposure of tumor cells to chemotherapeutic agents. In accordance with the present invention, Killer expression is assessed in conjunction with p53 status. These methods will facilitate identification of efficacious chemotherapeutic agents as measured by the induction of apoptosis in the targeted tumor cell.

Various terms relating to the biological molecules of the present invention are used hereinabove and also throughout the specifications and claims. The terms "substantially the same," "percent similarity" and "percent identity (identical)" are defined in detail in the description set forth below.

With reference to nucleic acids of the invention, the term "isolated nucleic acid" is sometimes used. This term, when applied to DNA, refers to a DNA molecule that is separated from sequences with which it is immediately contiguous (in the 5' and 3' directions) in the naturally occurring genome of the organism from which it was derived. For example, the "isolated nucleic acid" may comprise a DNA molecule inserted into a vector, such as a plasmid or virus vector, or integrated into the genomic DNA of a prokaryote or eucaryote.

With respect to RNA molecules of the invention, the term "isolated nucleic acid" primarily refers to an RNA molecule encoded by an isolated DNA molecule as defined above. Alternatively, the term may refer to an RNA molecule that has been sufficiently separated from

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RNA molecules with which it would be associated in its natural state (i.e., in cells or tissues), such that it exists in a "substantially pure" form (the term "substantially pure" is defined below).

With respect to protein, the term "isolated protein" or "isolated and purified protein" is sometimes used herein. This term refers primarily to a protein produced by expression of an isolated nucleic acid molecule of the invention. Alternatively, this term may refer to a protein which has been sufficiently separated from other proteins with which it would naturally be associated, so as to exist in "substantially pure" form.

The term "substantially pure" refers to a preparation comprising at least 50-60% by weight the compound of interest (e.g., nucleic acid, oligonucleotide, protein, etc.). More preferably, the preparation comprises at least 75% by weight, and most preferably 90-99% by weight, the compound of interest. Purity is measured by methods appropriate for the compound of interest (e.g. chromatographic methods, agarose or polyacrylamide gel electrophoresis, HPLC analysis, and the like).

With respect to antibodies of the invention, the term "immunologically specific" refers to antibodies that bind to one or more epitopes of a protein of interest (e.g., "Killer"), but which do not substantially recognize and bind other molecules in a sample containing a mixed population of antigenic biological molecules.

With respect to oligonucleotides, the term "specifically hybridizing" refers to the association between two single-stranded nucleotide molecules of sufficiently complementary sequence to permit such hybridization under pre-determined conditions generally used in the art (sometimes termed "substantially complementary"). In particular, the term refers to hybridization of an oligonucleotide with a substantially complementary sequence contained within a single-stranded

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DNA or RNA molecule of the invention, to the substantial exclusion of hybridization of the oligonucleotide with single-stranded nucleic acids of non-complementary sequence.

5 The nucleic acids, proteins and antibodies of the present invention are useful as diagnostic and therapeutic agents for the detection and treatment of cancer and other proliferative diseases. They should also find utility as research tools and will facilitate
10 the elucidation of the mechanistic action of the novel genetic and protein interactions involved in the control of chemotherapy induced cell death.

BRIEF DESCRIPTION OF THE DRAWINGS

15 Fig. 1 depicts the nucleic acid sequence of *Killer*, a novel adriamycin-inducible transcript. The figure shows the encoding nucleotide (SEQ ID NO:1) and predicted protein translation (SEQ ID NO:2) of the *Killer* open reading frame. The death domain is enclosed in a
20 box, and the putative transmembrane domain and signal peptide sequences are underlined by double and single lines, respectively. The termination codon is indicated by a star. *Killer* has a perfect Kozak sequence upstream of the initiating methionine 5'-CCGCCATGG-3'.

25 Figs. 2A and 2B show protein sequence homology between *Killer* and related proteins. Amino acid homology between *Killer* and its closest homolog DR4 (SEQ ID NO:3) is depicted in Fig. 2A. In the case of DR4, only the predicted mature protein sequence is shown (Pan et al.,
30 1997, *supra*). Identity and similarity are indicated by a star and a colon, respectively. The pairwise alignment was carried out using the MacVector™ 6.0 Clustal W(1.4) program (Oxford Molecular Group). Fig. 2B indicates the homology in the death domain regions of *Killer* and other
35 TNF receptor family members. A multiple sequence alignment algorithm was carried out using the MacVector™ 6.0 Clustal W(1.4) program (Oxford Molecular Group). A

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predicted death domain consensus is shown below the alignment. The degree of greyness reflects the degree of conservation between different family members. The following amino acids were included within the death domain of each family member: Killer aa 324-393 (part of SEQ ID NO:2); DR4 aa 356-404 (part of SEQ ID NO:3), DR3 aa 346-392 (SEQ ID NO: 4), Fas/apo1 aa 228-277 (SEQ ID NO:5), TNFR-1 aa 330-380 (SEQ ID NO:6), and CAR 1 aa 315-333 (SEQ ID NO:7).

10 **Figs. 3A, 3B and 3C** show regulation of *Killer* gene expression by wild-type p53. Fig. 3A: Northern analysis of *Killer* expression (upper panels) was carried out using RNA derived from different human cancer cell lines (as indicated) either untreated (-) or 10 hrs after 15 continuous treatment (+) with an apoptosis-inducing concentration 0.3 µg/ml of doxorubicin (adriamycin, adria) or 500 Rads ionizing radiation. Fig. 3B: Northern analysis shows that *Killer* mRNA expression is increased in H460 lung cancer cells 10 hours after continuous 20 treatment with 0.3 µg/ml doxorubicin (adriamycin), 5 µM Etoposide or a dose of 2,000 rads ionizing radiation at time zero. Fig. 3C: Northern analysis showing induction of *Killer* mRNA expression in mutant p53-expressing colon, ovarian or breast cancer cells 12 hours after infection 25 by either Ad-LacZ or Ad-p53. An ethidium stain of the RNA (lower panels) indicates equivalent RNA loading (10 µg) of the lanes in each experiment, and the p53 status of each cell line is shown below the ethidium stains (Figs 3A, 3C).

30 **Fig. 4** is a graph illustrating that expression of the death domain in *E. coli* is toxic. Bacteria were transformed with pGEX-GD vector encoding the death domain of Killer in the presence (open squares, open circles) or absence (closed squares, closed circles) of glucose. 35 Induction of death domain expression by IPTG resulted in a dramatic decrease in absorbance which was unaffected by the presence or absence of glucose ruling out a

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catabolite repression effect.

Fig. 5 shows that Killer is not induced in p53-dependent G1 arrest. Fig 5A: to induce p53-dependent cell cycle arrest in the absence of DNA damage, GM cells carrying a dexamethasone-inducible wild-type p53 exogenous transgene were incubated in the absence (lane 1) or presence (lanes 2-4) of dexamethasone. After incubation for increasing periods of time (as indicated) total RNA was isolated and Northern blot analysis, using either the human Killer (upper panel) or the human p21 (middle panel) cDNA as probes, was performed as described in Materials and Methods. An ethidium bromide stain of the RNA is shown (lower panel) to document equivalent loading of the gel. Fig. 5B: to induce a DNA damage/p53-dependent cell cycle arrest, WI38 cells were exposed to either 2 Gy or 10 Gy g-radiation. Total RNA was harvested at 10 hrs and Northern blotting performed as in A using either the Killer (upper panel) or the p21 (middle panel) cDNA as a probe. An ethidium bromide stain of the RNA is shown (lower panel) to document equivalent loading of the gel.

Figs. 6A and 6B show expression of Killer in cells undergoing apoptosis following Ad-p53 infection. Fig. 6A: expression of Killer is increased in GM cells undergoing apoptosis but not growth arrest. Northern blots of Killer mRNA expression in GM cells that were untreated (lane 1), treated with dexamethasone to induce a p53-dependent cell cycle arrest (lane 2), infected by Ad-LacZ as a control (lane 3) or infected by Ad-p53 (lane 4). An ethidium bromide stain is shown (below; 28S and 18S RNA indicated by arrows) to document equivalent loading of the RNA samples. Fig. 6B: p53-dependent upregulation of Killer mRNA expression occurs early in SKOV3 cells and continues for the next 48 hrs. Northern analysis of total RNA harvested at increasing time points (as indicated) following infection of SKOV3 cells by Ad-LacZ (lanes 2, 4, 6, 8, 10) or Ad-p53 (lanes 3, 5, 7,

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9, 11) for Killer expression.

Figs. 7A and 7B show transcriptional control of TRAIL death receptor expression in p53-dependent apoptosis. The human ovarian carcinoma cell line SKOV3 (Fig. 7A) or the human glioblastoma cell line GM (Fig. 7B) were infected by Ad-LacZ (lanes 1,2) or Ad-p53 (lanes 3,4). Following infection for one hour, cells were incubated either in the absence ("—" as indicated; lanes 1,3) or in the presence ("+" as indicated; lanes 2,4) of 30 ng/ml Actinomycin D for an additional 20 hrs when total cellular RNA was harvested for northern blotting for expression of Killer, p21, MDM2, and p53 mRNA (as indicated). An ethidium stain of the RNA is shown (lowest panels) to document equivalent loading of the gel.

Fig. 8 is a diagram of the physical map of the coding region of the human Killer gene. Exons are shown as black boxes; the number of the exon ("e") together with its size (in kb) are indicated above the boxes. The introns are shown as clear boxes and their respective sizes (in kb) are written within. The double headed arrows below represent the products of PCR reactions, shown together with their respective sizes. The translation initiation codon is depicted with a bent arrow, and the translation stop codon with a blunted arrow. The genomic position of the signal sequence (SS), the transmembrane domain (TM) and the death domain (DD) are indicated above the bar. The positions and the number of the extracellular Cysteine residues (C) are drawn above the bar. The gray boxes represent the position of clones obtained through subcloning P1-Killer, PstI digestion fragments (hybridizing to probes-A and B on Southern blots, shown above the boxes), into pBluescript.

Fig. 9 is the nucleotide sequence of a genomic clone of Killer, "P1-Killer" (SEQ ID NO:14). The putative CAT and TATA boxes are indicated, as is the putative start codon.

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DETAILED DESCRIPTION OF THE INVENTION

5 As described above, p53 plays a pivotal and complex role in preventing malignant transformation. In some instances, p53 has been found to activate apoptosis after treatment of cells with chemotherapeutic agents or radiation. However, the relationship between p53 status and sensitivity or resistance to such treatments remains 10 unclear, making it difficult to design or implement therapeutic strategies based on p53. Identification of downstream targets of p53 function in apoptotic signaling would help overcome this difficulty. One such target has 15 now been identified in accordance with the present invention.

Using a subtractive hybridization technique, the inventors have identified a unique cDNA clone corresponding to a gene whose expression is upregulated 20 in Adriamycin-treated chemosensitive teratocarcinoma cells. The pattern of inducibility of the gene product correlates well with the p53 status of the cell lines, indicating that expression of the gene is regulated by p53. The cloning and analysis of that cDNA is described 25 in detail below.

From the deduced amino acid sequence of the cDNA, it has been determined that the gene encodes a protein that is a new member of the tumor necrosis factor receptor (TNFR) protein family. This protein is referred 30 to herein as "Killer" or "Killer protein", in view of its presumed role in induction of apoptosis. The genomic DNA or cDNA that encodes the Killer protein is sometimes referred to herein as the "killer" gene or cDNA.

The TNFR family includes two TNFRs (TNFR1 and 35 TNFR2), the receptor for lymphotoxin- β , nerve growth factor (NGF) receptor (p75), CD40, CD27, CD30, DR3 (death receptor-3), DR4, HVEM (herpes virus early mediator), and

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CAR1 (cytopathic avian leukosis-sarcoma virus receptor. See Beutler, B. & Van Huffel, *Ann. NY Acad. Sci.*, 730:118-133 (1994), S. Nagata, *Cell*, 88:355-365 (1997), G. Pan, et al., *Science*, 276:111-113 (1997). The Killer protein of the invention shares general structural features of the TNFR family in that it is believed to comprise three domains: (1) an extracellular domain at the amino terminus; (2) a transmembrane domain in the central region; and (3) a carboxyl "death" domain. Functionally, Killer is believed to behave in general like a TNFR: the extracellular domain functions as receptor for a cognate extracellular ligand (analogous to TNF for TNFR1); binding of the ligand to the extracellular receptor domain initiates a conformational change through the transmembrane domain to the death domain, which transduces a signal (or cascade of signals) within the cell that ultimately induces apoptosis.

A comparison of Killer with the closely related members of the TNFR (specifically focusing on the death domain) family is set forth in greater detail in Example 1 and shown in Figures 1B and 2. As can be seen, though Killer shares sufficient amino acid sequence similarity with other members of the TNFR family to warrant including it within that family, it is a unique member of that family. The closest homolog is DR4, which shares only about 64% amino acid sequence identity with Killer, even in the most conserved death domain, and about 60% identity comparing all of SEQ ID NO:2, which comprises the death domain, the transmembrane domain and the extracellular domain. Moreover, expression of the Killer gene appears to be regulated by p53, presumably through one or more p53 binding sites. The gene that encodes DR4 has not been reported to be regulated by p53.

Killer also appears to be unique in that it is regulated by DNA damage. Results obtained by the present inventor demonstrate that, whereas Killer mRNA expression is induced by DNA damage in wild-type p53 containing

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cells, so such universal phenomenon was observed for either DR4 or the decoy receptor, TRID.

Loss of Killer's apoptotic function has also been linked to a human cancer. Transfections of head and neck squamous cell carcinoma, colon and ovarian carcinoma cell lines revealed loss of growth-suppressive function associated with a Killer truncation mutant derived from head and neck tumor.

Example 2 describes further important characteristics of Killer. In that Example it is shown that, unlike the p53-target CDK-inhibitor *p21*^{WAF1/CIP1}, Killer is only induced in cells undergoing p53-dependent apoptosis and not cell cycle arrest. Inhibition of transcription by Actinomycin D blocks both Killer and *p21* gene induction in cells undergoing p53-dependent apoptosis, indicating that p53 regulation of Killer is transcriptional.

The DNA of the invention encodes a Killer protein. The gene, its corresponding cDNA and encoded protein have been designated Killer due to its involvement in the apoptotic pathway. The gene is turned on early in the dying process in chemotherapy treated chemosensitive, but not chemoresistant ovarian cancer cells. Apoptosis is a type of cell death that is thought to be under direct genetic control. During apoptosis, cells lose their cell junctions and microvilli. The cytoplasm condenses and nuclear chromatin marginates into a number of discrete masses. While the nucleus fragments, the cytoplasm contracts and mitochondria and ribosomes become densely compacted. After dilation of the endoplasmic reticulum and its fusion with the plasma membrane, the cell breaks up into several membrane bound vesicles, also known as apoptotic bodies, which are usually phagocytosed by adjacent cells.

The death domain of Killer is highly homologous to such domains in other known death receptors that cause apoptosis in response to a variety of other signals. In

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accordance with the present invention, it has also been shown that the expression of the death domain in *E. coli* is toxic.

Figure 1 sets forth the nucleotide sequence (SEQ ID NO:1) and deduced amino acid sequence (SEQ ID NO:2) of a cDNA of human origin encoding Killer protein of the invention. SEQ ID NO:1 comprises the complete coding sequence of Killer. The protein contains a transmembrane domain, a C-terminal death domain, and an N-terminal extracellular domain. This sequence information indicates that Killer may be classified as a new member of the TNFR family, as discussed above.

Although the human Killer-encoding gene is described and exemplified herein, the present invention also encompasses nucleic acid sequences and proteins from other species that are functionally and structurally homologous to be used interchangeably with Killer-encoding nucleic acids and proteins for the diagnostic, therapeutic, and research purposes described below. Because of the high degree of conservation of genes encoding specific signal transducers and related oncogenes, it will be appreciated by those skilled in the art that, even if the interspecies Killer similarity is low, Killer-encoding nucleic acids and Killer proteins from a variety of mammalian species should possess a sufficient degree of homology with Killer so as to be interchangeably useful with Killer in such diagnostic and therapeutic applications. Accordingly, the present invention is drawn to mammalian Killer-encoding nucleic acids and Killer proteins, preferably to Killer of primate origin, and most preferably to Killer of human origin. Accordingly, when terms such as "Killer" or "Killer-encoding nucleic acid" are used herein, they are intended to encompass mammalian Killer-encoding nucleic acids and Killer proteins falling within the confines of homology set forth below, of which human Killer is an exemplary member.

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Allelic variants and natural mutants of SEQ ID NO:1 are likely to exist within the human genome and within the genomes of other mammalian species. Because such variants are expected to possess certain differences 5 in nucleotide and amino acid sequence, this invention provides an isolated nucleic acid molecule and an isolated Killer protein having at least about 50-60% (preferably 60-80%, most preferably over 80%) sequence homology in the coding region with the nucleotide 10 sequence set forth as SEQ ID NO:1, and the amino acid sequence of SEQ ID NO:2. Such nucleic acid sequences will also specifically hybridize with SEQ ID NO:1 or its complement.

Because of the natural sequence variation 15 likely to exist among Killer proteins and nucleic acids encoding them, one skilled in the art would expect to find up to about 40-50% sequence variation, while still maintaining the unique properties of the Killer protein of the present invention. Such an expectation is due in 20 part to the degeneracy of the genetic code, as well as to the known evolutionary success of conservative amino acid sequence variations, which do not appreciably alter the nature of the protein. Accordingly, such variants are considered substantially the same as one another and are 25 included within the scope of the present invention.

For purposes of this invention, the term "substantially the same" refers to nucleic acid or amino acid sequences having sequence variations that are conservative or neutral. With particular reference to 30 nucleic acid sequences, the term "substantially the same" is intended to refer to the coding region and to conserved sequences governing expression, and refers primarily to degenerate codons encoding the same amino acid, or alternate codons encoding conservative 35 substitute amino acids in the encoded polypeptide. With reference to amino acid sequences, the term "substantially the same" refers generally to conservative

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substitutions and/or variations in regions of the polypeptide. The terms "percent identity" and "percent similarity" are also used herein in comparisons among amino acid sequences. These terms are intended to be 5 defined as they are in the UWGCG sequence analysis program (Devereaux et al., Nucl. Acids Res. 12: 387-397, 1984), available from the University of Wisconsin.

The following description sets forth the general procedures involved in practicing the present 10 invention. To the extent that specific materials are mentioned, it is merely for purposes of illustration and is not intended to limit the invention. Unless otherwise specified, general cloning procedures, such as those set forth in Sambrook et al., Molecular Cloning, Cold Spring 15 Harbor Laboratory (1989) (hereinafter "Sambrook et al.") are used.

20 I. Preparation of Killer-Encoding Nucleic Acid Molecules, Killer Proteins and Antibodies Immunologically Specific for those Proteins

A. Nucleic Acid Molecules

25 Nucleic acid molecules encoding the Killer proteins of the invention may be prepared by two general methods: (1) they may be synthesized from appropriate nucleotide triphosphates, or (2) they may be isolated from biological sources. Both methods utilize protocols well known in the art.

30 The availability of nucleotide sequence information, such as the cDNA having SEQ ID NO:1, enables preparation of an isolated nucleic acid molecule of the invention by oligonucleotide synthesis. Synthetic oligonucleotides may be prepared by the phosphoramidite 35 method employed in the Applied Biosystems 38A DNA Synthesizer or similar devices. The resultant construct may be purified according to methods known in the art, such as high performance liquid chromatography (HPLC). Long, double-stranded polynucleotides, such as a DNA

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molecule of the present invention, must be synthesized in stages, due to the size limitations inherent in current oligonucleotide synthetic methods. Thus, for example, a 4.44 kb double-stranded molecule may be synthesized as several smaller segments of appropriate complementarity. 5 Complementary segments thus produced may be annealed such that each segment possesses appropriate cohesive termini for attachment of an adjacent segment. Adjacent segments may be ligated by annealing cohesive termini in the presence of DNA ligase to construct an entire double- 10 stranded molecule. A synthetic DNA molecule so constructed may then be cloned and amplified in an appropriate vector.

Nucleic acid sequences encoding Killer may be isolated from appropriate biological sources using 15 methods known in the art. In a preferred embodiment, a cDNA clone is isolated by subtractive hybridization of a cDNA library of human origin. In an alternative embodiment, human genomic clones encoding Killer may be isolated. 20 In fact, genomic clones of Killer have been isolated (an exemplary clone, P1-Killer, is shown in Fig. 9), and a complete physical map of the Killer gene, located on human chromosome 8p21, has been constructed (Figure 8). Alternatively, Killer cDNA or genomic clones 25 from other mammalian species may be obtained.

In accordance with the present invention, nucleic acids having the appropriate level sequence homology with a selected region of SEQ ID NO:1 (e.g. the segment encoding the transmembrane domain or the death 30 domain) may be identified by using hybridization and washing conditions of appropriate stringency. For example, hybridizations may be performed, according to the method of Sambrook et al., using a hybridization solution comprising: 5X SSC, 5X Denhardt's reagent, 1.0% 35 SDS, 100 µg/ml denatured, fragmented salmon sperm DNA, 0.05% sodium pyrophosphate and up to 50% formamide. Hybridization is carried out at 37-42°C for at least six

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hours. Following hybridization, filters are washed as follows: (1) 5 minutes at room temperature in 2X SSC and 1% SDS; (2) 15 minutes at room temperature in 2X SSC and 0.1% SDS; (3) 30 minutes-1 hour at 37°C in 1X SSC and 1% SDS; (4) 2 hours at 42-65° in 1X SSC and 1% SDS, changing the solution every 30 minutes.

One common formula for calculating the stringency conditions required to achieve hybridization between nucleic acid molecules of a specified sequence homology (Sambrook et al., 1989):

$$T_m = 81.5^\circ\text{C} + 16.6 \log [\text{Na}^+] + 0.41(\% \text{ G+C}) - 0.63 (\% \text{ formamide}) - 600/\#\text{bp in duplex}$$

As an illustration of the above formula, using $[\text{N}^+] = [0.368]$ and 50% formamide, with GC content of 42% and an average probe size of 200 bases, the T_m is 57°C. The T_m of a DNA duplex decreases by 1 - 1.5°C with every 1% decrease in homology. Thus, targets with greater than about 75% sequence identity would be observed using a hybridization temperature of 42°C. Such sequences would be considered substantially complementary to the probe.

Nucleic acids of the present invention may be maintained as DNA in any convenient cloning vector. In a preferred embodiment, clones are maintained in plasmid cloning/expression vector, such as pBluescript (Stratagene, La Jolla, CA), which is propagated in a suitable *E. coli* host cell. Optionally, the vectors may contain inducible promoter sequences, such as metallothioneine, tetracycline or dexamethasone responsive promoters. Such vectors enable the investigator to regulate the expression of killer protein in the transformed tumor cell while assessing induction of apoptosis.

Killer-encoding nucleic acid molecules of the invention include cDNA, genomic DNA, RNA, and fragments thereof which may be single- or double-stranded. Thus, this invention provides oligonucleotides (sense or

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antisense strands of DNA or RNA) having sequences capable of hybridizing with at least one sequence of a nucleic acid molecule of the present invention, such as selected segments of the cDNA having SEQ ID NO:1. Such 5 oligonucleotides are useful as probes for detecting Killer genes in test samples of potentially malignant cells or tissues, e.g. by PCR amplification, or for the isolation of homologous regulators of apoptosis.

In addition, 5' and 3' regulatory sequences of 10 Killer, available on the genomic clone discussed above, can be used to advantage. For instance, the promoter sequence can be cloned upstream of a reporter gene, so that drug screening can be performed to isolate compounds that induce Killer's expression. Reporters may include 15 LacZ, Luciferase, GFP, Hygromycin, or other negative or positive selection markers.

B. proteins

A Killer protein of the present invention may 20 be prepared in a variety of ways, according to known methods. The protein may be purified from appropriate sources, e.g., human or animal cultured cells or tissues, by immunoaffinity purification. However, this is not a preferred method due to the low amount of protein likely 25 to be present in a given cell type at any time.

The availability of nucleic acids molecules encoding Killer enables production of the protein using 30 in vitro expression methods known in the art. For example, a cDNA or gene may be cloned into an appropriate in vitro transcription vector, such a pSP64 or pSP65 for in vitro transcription, followed by cell-free translation in a suitable cell-free translation system, such as wheat germ or rabbit reticulocytes. In vitro transcription and 35 translation systems are commercially available, e.g., from Promega Biotech, Madison, Wisconsin or BRL, Rockville, Maryland.

Alternatively, according to a preferred

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embodiment, larger quantities of Killer may be produced by expression in a suitable prokaryotic or eukaryotic system. For example, part or all of a DNA molecule, such as the cDNA having SEQ ID NO:1 may be inserted into a 5 plasmid vector adapted for expression in a bacterial cell, such as *E. coli*, or into a baculovirus vector for expression in an insect cell. Such vectors provide the regulatory elements necessary for expression of the DNA in the given host cell, positioned in such a manner as to permit expression of the DNA in the host cell. Such 10 regulatory elements required for expression include promoter sequences, transcription initiation sequences and, optionally, enhancer sequences.

The Killer protein produced by gene expression 15 in a recombinant prokaryotic or eukaryotic system may be purified according to methods known in the art. In a preferred embodiment, a commercially available expression/secretion system can be used, whereby the recombinant protein is expressed and thereafter secreted 20 from the host cell, to be easily purified from the surrounding medium. If expression/secretion vectors are not used, an alternative approach involves purifying the recombinant protein by affinity separation, such as by immunological interaction with antibodies that bind 25 specifically to the recombinant protein. Such methods are commonly used by skilled practitioners.

The Killer proteins of the invention, prepared by the aforementioned methods, may be analyzed according to standard procedures. For example, such proteins may 30 be subjected to amino acid sequence analysis, according to known methods.

The present invention also provides antibodies 35 which bind immunospecifically to Killer proteins may be prepared according to standard methods. In a preferred embodiment, monoclonal antibodies are prepared, which react immunospecifically with various epitopes of Killer. Monoclonal antibodies may be prepared according to

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general methods of Köhler and Milstein, following standard protocols. Polyclonal or monoclonal antibodies that immunospecifically interact with Killer can be utilized for identifying and purifying such proteins.

5 For example, antibodies may be utilized for affinity separation of proteins with which they immunospecifically interact. Antibodies may also be used to immunoprecipitate proteins from a sample containing a mixture of proteins and other biological molecules.

10 Other uses of anti-Killer antibodies are described below.

15

II. Uses of Killer-Encoding Nucleic Acids, Killer Proteins and Antibodies Thereto

Cellular signaling molecules, including proteins involved in apoptosis pathways, have received a great deal of attention as potential mediators of selective killing of tumor cells for the elimination of cancer from the body. As a signaling molecule involved in p53-mediated apoptosis, Killer and related proteins from other mammalian species will be particularly useful as diagnostic and therapeutic agents. Such molecules will also provide valuable research tools.

A. Killer-Encoding Nucleic Acids

Killer-encoding nucleic acids may be used for a variety of purposes in accordance with the present invention. Killer-encoding DNA, RNA, or fragments thereof may be used as probes to detect the presence of and/or expression of genes encoding Killer, as well as to identify alterations of killer, resulting in loss of function, as they arise in various cancers. In this regard, it should be noted that the diagnostic value of detecting Killer expression, even at the mRNA level, is significant. Killer DNA may be hypermethylated or simply not activated after exposure to chemotherapy in tumors that go on to become treatment-resistant. Such

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information may be useful in designing chemotherapy for particular patients. Methods in which Killer-encoding nucleic acids may be utilized as probes for such assays include, but are not limited to: (1) *in situ* hybridization; (2) Southern hybridization (3) northern hybridization; and (4) assorted amplification reactions such as polymerase chain reactions (PCR).

The Killer-encoding nucleic acids of the invention may also be utilized as probes to identify related genes either from humans or from other species. As is well known in the art, hybridization stringencies may be adjusted to allow hybridization of nucleic acid probes with complementary sequences of varying degrees of homology. Thus, Killer-encoding nucleic acids may be used to advantage to identify and characterize other genes of varying degrees of relation to Killer, thereby enabling further characterization of the signaling cascade involved in the control of apoptosis in different cell types. Additionally, they may be used to identify genes encoding proteins that interact with Killer (e.g., by the "interaction trap" technique), which should further accelerate elucidation of these cellular signaling mechanisms (Golemis, et al., *Interaction trap/two-hybrid systems to identify interacting proteins*, Unit 20.1.1-20.1.28 in *Current Protocols in Molecular Biology*, eds. F.M. Ausubel, et al. John Wiley & Sons, NY (1996)). Yeast two-hybrid screening may be used to identify the extracellular ligand(s) for Killer, and the proteins that interact with the cytoplasmic portions of Killer, including death domain-containing protein partners. Killer may function to promote apoptosis through a pathway analogous to the Fas and TNFR1 systems. The Fas apoptosis pathway is activated by the binding of Fas ligand (FasL) to Fas, and TNF-induced apoptosis begins by ligation of TNF to TNFR1. The use of interaction trap/two-hybrid assays will permit the identification of Killer-specific, extracellular ligands.

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which trigger the Killer apoptosis cascade. Further, these assays will be used to identify the intracellular proteins that interact with Killer to potentiate the signal within the cell.

5 Nucleic acid molecules, or fragments thereof, encoding Killer may also be utilized to control the expression of Killer, thereby regulating the amount of protein available to participate in apoptosis signaling pathways. Alterations in the physiological amount of
10 Killer protein may act synergistically with chemotherapeutic agents used to treat cancer.

In one embodiment, the nucleic acid molecules of the invention may be used to decrease production of Killer protein in a population of cells. In this 15 embodiment, Killer proteins would be made unavailable for the transduction of specific apoptosis signals, providing a model system for research purposes. In this embodiment, antisense oligonucleotides are employed which are targeted to specific regions of Killer-encoding genes 20 that are critical for gene expression. The use of antisense oligonucleotides to decrease expression levels of a pre-determined gene is known in the art. In a preferred embodiment, such antisense oligonucleotides are modified in various ways to increase their stability and 25 membrane permeability, so as to maximize their effective delivery to target cells *in vitro* and *in vivo*. Such modifications include the preparation of phosphorothioate or methylphosphonate derivatives, among many others, according to procedures known in the art. Antisense 30 molecules may also be introduced on conventional DNA vectors used for gene therapy. Once in the appropriate target cells, the antisense molecule is produced by expression of a recombinant DNA segment under control of appropriate promoters and other regulatory sequences.

35 In another embodiment designed to decrease Killer expression or activity, inhibitors of such expression or activity are identified and utilized. Such

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inhibitors would be useful not only for laboratory studies, but also therapeutically, as agents that protect normal tissue against the cytotoxic effects of chemotherapy. A similar approach is being taken, for 5 example, using expression of the MDR1 glycoprotein in bone marrow cells. The MDR1 glycoprotein has been found to be overexpressed in tumor cells that have become resistant to chemotherapeutic agents. Inhibitors of MDR1 are useful to counteract this effect. Similarly, if 10 Killer mutates in any tumors and such mutant Killer interferes with cell death pathwayss, then inhibitors of Killer would be of direct therapeutic value in such cases.

In another embodiment, overexpression of Killer 15 is induced in a target population of cells to generate an excess of Killer molecules. This excess production of Killer may be monitored with immunofluorescence or any other standard technique known in the art. Further, excess production of Killer protein will trigger 20 apoptosis and facilitate the isolation and characterization of the intracellular death domain-containing proteins that interact with Killer and potentiate the apoptosis signal.

Another embodiment of the invention is excess 25 production of the Killer death domain in target cells to assess the effect of the death domain on apoptosis signaling and chemosensitivity.

Another use of the nucleic acids of the invention is in gene replacement therapy for expression 30 of Killer in instances of null or mutant p53 status in a particular target tumor, such that apoptosis may be triggered in tumor cells that will not die in response to chemotherapy. Gene therapy to provide a functional Killer to cells lacking the functional protein is also 35 contemplated. This sort of gene therapy should prove particularly useful in cancers (such as the head and neck cancer described above) specifically associated with a

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disrupted Killer gene.

As described above, Killer-encoding nucleic acids are also used to advantage to produce large quantities of substantially pure Killer protein, or selected portions thereof. In a preferred embodiment, 5 the C-terminal death domain of Killer (see Figure 1B) is produced by expression of a nucleic acid encoding the domain. The full-length protein or selected domain is thereafter used for various research, diagnostic and 10 therapeutic purposes, as described below.

B. Killer Protein and Antibodies

Purified Killer, or fragments thereof, may be used to produce polyclonal or monoclonal antibodies which 15 also may serve as sensitive detection reagents for the presence and accumulation of Killer (or complexes containing Killer) in cultured cells or tissues from living patients (the term "patients" refers to both humans and animals). Recombinant techniques enable 20 expression of fusion proteins containing part or all of the Killer protein. The full length protein or fragments of the protein may be used to advantage to generate an array of monoclonal antibodies specific for various epitopes of the protein, thereby providing even greater 25 sensitivity for detection of the protein in cells or tissue.

Polyclonal or monoclonal antibodies immunologically specific for Killer may be used in a variety of assays designed to detect and quantitate the 30 protein, which may serve as a prognostic indicator for malignant disease. Such assays include, but are not limited to: (1) flow cytometric analysis; (2) immunochemical localization in Killer in cultured cells or biopsy tissue; and (3) immunoblot analysis (e.g., dot blot, Western blot) of extracts from various cells and 35 tissues. Additionally, as described above, anti-Killer antibodies can be used for purification of Killer (e.g.,

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affinity column purification, immunoprecipitation).

Anti-Killer antibodies may also be utilized as therapeutic agents to block the normal functionality of Killer in a target cell population. Thus, similar to the 5 antisense oligonucleotides described above, anti-Killer antibodies may be delivered to a target cell population by methods known in the art (i.e. through various lipophilic carriers that enable delivery of the compound of interest to the target cell cytoplasm) where the 10 antibodies may interact with intrinsic Killer protein to render it nonfunctional.

Alternatively, the possibility exists that anti-Killer antibodies may in fact activate Killer's receptor and have a therapeutic value in inducing 15 apoptosis. Precedent exists for such a function, for instance, with anti-Fas antibodies. An anti-Killer antibody use for such a purpose may prove superior to the true ligand, and it may be deliverable intravenously, depending on its specificity for cancer cells, and 20 absence of toxicity. The antibody also may be injected directly into a tumor.

From the foregoing discussion, it can be seen that Killer-encoding nucleic acids and antibodies to 25 Killer proteins of the invention can be used to detect gene expression and protein accumulation for purposes of assessing the genetic and protein interactions involved in the regulation of apoptosis in both wild-type p53 positive and negative cells.

Aberrant signal transduction in cells is often 30 correlated with cellular transformation and cancer of various tissue types. It is expected that these tools will be particularly useful for diagnosis and prognosis of human neoplastic disease as described above. Potentially of greater significance, however, is the 35 utility of Killer-encoding nucleic acids, proteins and antibodies as agents to assess and alter the chemosensitivity of a targeted tumor cell. In any of

their therapeutic uses, the nucleic acids, proteins and antibodies of the invention can be used alone, or as adjuncts to other therapeutic agents.

Although the compositions of the invention have been described with respect to human diagnostics and therapeutics, it will be apparent to one skilled in the art that these tools will also be useful in animal and cultured cell experimentation with respect to various malignancies and/or other conditions manifested by altered patterns of apoptosis or lack of apoptosis in response to DNA damaging agents or ionizing radiation. They can also be used to generate animal model systems, e.g. "knockout" animals that do not express the gene, as a model of cancer susceptibility. Such animals are useful as models for human disease and treatment thereof, inasmuch as they may exhibit phenotypes that may mimic a developmental abnormality and may have a predisposition to cancer, especially following exposure to carcinogens. In connection with this embodiment, it is preferable to clone a mouse homolog of Killer, according to standard methods as described above.

The following Examples are provided to describe the invention in further detail. The examples are intended to illustrate and not to limit the invention.

EXAMPLE 1
Isolation and Characterization of a Nucleic Acid
Molecule Encoding Human Killer Protein

In this Example the cloning of a cDNA molecule encoding human Killer is described. To identify genes transcriptionally regulated by wild-type p53 in response to DNA damage, a cDNA library enriched for the presence of such genes was constructed and screened by a subtractive hybridization technique. The cell line used as a source for the cDNA library was PA-1, a human, wild-type p53-positive, ovarian teratocarcinoma. p53 status

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correlates with chemosensitivity in these cells (Wu & El-Deiry, *Nature Med.* 2: 255-256, 1996).

Materials and Methods

5 Cell lines and culture conditions. The human leukemia cell line ML-1, a gift from Michael B. Kastan (Johns Hopkins University, Baltimore, MD), the human lung cancer cell line H460, a gift from Stephen B. Baylin (Johns Hopkins University, Baltimore, MD) and the human colon carcinoma cell line HCT-116, a gift from Dr. Bert Vogelstein (Johns Hopkins University, Baltimore, MD) were maintained in RPMI 1640 supplemented with 10% fetal bovine serum (FBS), 1% penicillin and streptomycin (Wu et al., *Clin. Cancer Res.* 2: 623-633, 1996; Prabhu et al., *Clin. Cancer Res.* 2: 1221-1229, 1996). The human ovarian cancer cell lines PA-1 and SKOV3, and the human breast cancer line SKBr3 were obtained from the American Type Culture Collection (Rockville, MD) and maintained as previously described (Wu et al., 1996, *Supra*; Blagosklonny & El Deiry, *Int. J. Cancer* 67: 386-397, 1996). The human colon carcinoma cell line SW480 was obtained from the University of Pennsylvania Cell Center and maintained as previously described (Fuchs et al., *Cancer Res.* 57: 2550-2554, 1997). HPV-E6 expressing clones of HCT-116, PA-1, and H460 were transfected and maintained as previously described (Wu & El Deiry, *Nature Med.* 2: 255-256, 1996; Wu et al., 1996, *supra*; Prabhu et al., 1996, *supra*). Cells were grown in the presence of 0.3 µg/ml adriamycin for 10 hrs followed by isolation of total RNA as previously described (El Deiry et al., *Cell* 75: 817-825, 1993). H460-neo and H460-E6 cells were irradiated at 500 rads and grown for 10 hrs followed by isolation of total RNA as previously described (Fuchs et al., 1997, *supra*; El Deiry et al., 1993, *supra*).
30 Replication-deficient adenovirus recombinants expressing β-galactosidase (Ad-LacZ) or wild-type p53 (Ad-p53) were gifts from Dr. B. Vogelstein (Johns Hopkins University),
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and were prepared, titered, and used as previously described (Fuchs et al., 1997, *supra*; Blagosklonny & El Deiry, 1996, *supra*; El Deiry et al., 1993, *supra*). Cell lines were infected at a multiplicity of infection (MOI) 5 of 50, and total protein and RNA were harvested 20 hrs later. The infectivity of the cell lines was determined by X-gal staining, as previously described (Blagosklonny & El Deiry, 1996, *supra*).

Subtractive hybridization screening. PA1-neo 10 ovarian teratocarcinoma cells (Wu & El Deiry, 1996, *supra*) were incubated for 10 hrs in the presence of 0.3 µg/ml adriamycin. A cDNA library was constructed using poly(A+) RNA prepared from the adriamycin-treated cells. A total of 50,000 plaques were differentially screened by 15 hybridization to the following: (1) a cDNA probe prepared from equal portions of RNA isolated from untreated PA1-neo cells and PA1-E6 cells, and (2) a subtracted probe prepared by using 500 ng cDNA from adriamycin-treated PA1-neo cells as target and a total of 20 µg driver poly (A+) RNA (10 µg from untreated PA1-neo 20 cells and 10 µg from adriamycin-treated PA1-E6 cells). The cDNA library and probes were prepared using previously described methods (El Deiry et al., 1993).

The initial isolate of the Killer cDNA was 0.6 25 kb of the extreme 3'-untranslated region and this hybridized to a 4.4 kb mRNA species. Hybridization of this probe to the original oligo dT-primed adriamycin-treated PA1 cDNA library yielded a 2.0 kb fragment containing 3'-untranslated Killer sequence with 30 no homology in the Genbank database. We performed 5'-RACE PCR to obtain the 5'-coding sequence of Killer using the Marathon™ cDNA Amplification Kit (Clontech).

Northern Blot Analyses. Northern blot analyses 35 were performed using either 10 µg of total cellular RNA (Figs. 1A,3) or 2 µg poly (A+) RNA (Multiple Tissue Northern (MTN™) Blot from Clontech) as previously described. The PA1-neo/E6 northern blot was probed with

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a 600 bp 3' untranslated region of the Killer cDNA. All other northern blots were probed with a 1.14 kb fragment from the C-terminal coding region of the Killer cDNA. In addition, a 2.0 kb human β -actin probe (Clontech) was used to probe the MTNTM blot. p21 mRNA expression was detected as previously described (Fuchs et al., 1996, *supra*; El Deiry et al., 1993, *supra*).

Plasmids. The full-length open reading frame of the Killer cDNA was amplified by RT-PCR from adriamycin-treated PA1 cellular RNA using 5'-CCCAAGCTTCCGCCATGGAACAAACGGGGAC-3' (SEQ ID NO:8) and 5'-CGCGGATCCTTAGGACATGGCAGAGTCTGC-3' (SEQ ID NO:9) and the following PCR conditions: 35 cycles of denaturation at 95°C for 30 seconds followed by annealing at 68°C for 90 seconds and extension at 72°C for 60 seconds. This PCR product was subcloned into the pCRII vector using the TA-cloning kit (Invitrogen) and following restriction by BamH1 and HindIII the cDNA was cloned into the mammalian expression vector pCEP4 (Invitrogen). The cytoplasmic domain was obtained by PCR amplification (30 cycles: 95°C for 30 sec, 65°C for 1 min, 72°C for 1 minute) using Killer cDNA as template and the following primers: 5'-CGCGGATCCGTCCATTTCTGCTTGG-3' (SEQ ID NO:10) and 5'-CCCAAGCTTCCACCATGGTTGCAGCCGTAGTCTTG-3'. (SEQ ID NO:11). The death domain was amplified using the primers 5'-CCCAAGCTTCCACCATGGACTCCTGGGAGCCGC-3' (SEQ ID NO:12) and 5'-CGCGGATCCGTCCATTTCTGCTTGG-3' (SEQ ID NO:13) and the same PCR conditions as the cytoplasmic domain. The PCR products were restricted with BamH1 and HindIII and subcloned into pCEP4. For *in-situ* RNA hybridization, nucleotides 106 to 726 of Killer (Figure 1) by PCR amplification and TA-cloning into the pCRTMII (Invitrogen) vector in either sense or antisense orientation.

Transfections and apoptosis assays.
Transfection of SW480 cells was carried out as previously described (El Deiry et al., 1993, *supra*). TUNEL assays

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were performed using SW480 cells transfected in 8-well chamber slides. After 36 hrs, TUNEL-positive cells were detected using the Apoptag Plus *In-situ* apoptosis detection kit-Fluorescein (ONCOR). Transfection efficiency in these experiments was 20%.

5 *Chromosomal localization.* Fluorescence *in-situ* hybridization (FISH) was performed by standard techniques as previously described (Krantz et al., Am. J. Med. Genet. 70: 80-86, 1997). Three P1 clones (Genome Systems) containing the Killer gene were labeled by nick 10 translation with biotin-11-dUTP and hybridized to metaphase spreads from a normal individual. Chromosomes were counter-stained with 4,6-diamino-2-phenyl-indole (DAPI). Analysis was carried out on a Vysis imaging system and after FISH, DAPI staining was computer 15 converted to a G-banding image for chromosome and band identification.

15 *In-situ mRNA expression.* *In-situ* hybridization of frozen sections was performed using a modified version 20 of the digoxigenin labeled method as described (Panoskaltsis-Mortari & Bucy, BioTech 18: 300-307, 1995). In brief, colonic tissue was snap frozen in OCT and stored at -80°C. Five µm sections were cut and placed on Probe-On slides (Fisher Scientific, Pittsburgh, PA). The 25 tissue sections were thawed at room temperature for 1-2 minutes and fixed in 3% paraformaldehyde for 1 minute at room temperature. After one wash with 2 x SSC, the slides were incubated for 8 minutes in 0.2 M HCl and rinsed with 0.1 M triethanolamine, pH 8.0. The slides 30 were then incubated in 0.25% acetic anhydride in 0.1 M triethanolamine, pH 8.0 for 15 minutes followed by a rinse with 2 x SSC. Pre-hybridization was performed at 35 50°C for 30 minutes in a solution containing 50% formamide, 4 x SSC, 1 x Denhardt's, 10% dextran sulfate, 500 µg/ml of heat denatured salmon sperm DNA, and 250 µg/ml of yeast tRNA. The slides were then hybridized in pre-hybridization solution containing heat-denatured

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(80 °C) RNA probe at 50 °C overnight. Following hybridization, the slides were rinsed in 2 x SSC for 5 minutes and STE (500 mM NaCl, 20 mM tris-HCl, pH 7.5, 1 mM EDTA) for 1 minute. After incubation in RNase A (40 µg/ml in STE) for 30 minutes at 37 °C, the slides were washed with 2 x SSC containing 50% formamide for 5 minutes at 50 °C followed by three washes with 1 x SSC and 0.5 x SSC, each for 5 minutes. Immunological detection of digoxigenin was carried out using a sheep-anti-digoxigenin antibody (Boehringer Mannheim, Indianapolis, IN). Digoxigenin labeled RNA probes for Killer (both sense and anti-sense) were transcribed from the T7 promoter of pCR™II (Invitrogen), after linearization by digestion with HindIII, using the Riboprobe Gemini System 2 kit (Promega) and the Dig/Genius™4 RNA Labeling Kit (SP6/T7; Boehringer Mannheim).

Targeting p53 for destruction. To generate a tumor cell line in which p53 function was lost, PA-1 cells were transfected with a human papilloma virus (HPV) 16 E6 expression plasmid that expresses E6 protein constitutively driven by the cytomegalovirus (CMV) immediate early promoter. PA-1 cells were transfected with either pCMV-neo-bam (referred to herein as either PA-1/neo or "wild-type") or pCMV16E6 (referred to herein as either PA-1/E6 or "degraded" to reflect the function of the E6 protein in degrading p53), as described previously (El-Deiry et al., 1993, *supra*). Individual clones were isolated following selection in the presence of 500 µg/ml G418 (Life Technologies, Inc.), as described by Baker et al., *Science* 249: 912-915 (1990).

Expression of the Death Domain of Killer in *E. coli*. *E. coli* cells were transformed with a pGEX vector expressing the death domain of the killer protein. Bacterial cells were seeded at low density and grown up overnight in L broth + ampicillin. SDS-PAGE was performed which confirmed that the protein was expressed

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following induction with IPTG. The toxicity assay was performed in the presence and absence of glucose to rule out catabolite repression effects. Toxicity of the death domain was assessed by measuring absorbance at 520 nM in 5 a spectrophotometer over a 5 hour time course.

Results

The *p53* tumor suppressor is the most commonly mutated gene in human cancer, and inherited mutations 10 lead to the cancer-prone Li-Fraumeni syndrome. There is evidence that *p53* is a key determinant of the sensitivity of mammalian cells to the cytotoxic effects of chemo- and radio-therapy. Biochemical studies have demonstrated that the majority of tumor-derived mutants of *p53* have 15 lost the ability to activate gene expression, suggesting that transcriptional activation by *p53* may be critical for its role in the control of cell proliferation. Stabilization of *p53* protein following virus infection, nucleotide depletion, hypoxia or DNA damage leads to cell 20 cycle arrest and/or apoptosis. Cell cycle arrest is believed to be mediated by transcriptional activation of *p21^{WAF1/CIP1}*, a universal inhibitor of cyclin-dependent kinases and PCNA-dependent processive DNA replication. *p53* upregulates expression of several genes involved in 25 apoptosis, including *bax* and *Fas/APO1*. However, the fact that neither *bax*- nor *Fas/APO1*-null cells are deficient in DNA damage-induced (*p53*-dependent) apoptosis suggests that other mechanisms likely contribute to this process.

30 We previously found that targeted degradation of the *p53* protein by HPV16 *E6* overexpression in PA1 ovarian teratocarcinoma cells led to inhibition of adriamycin- induced apoptosis and chemoresistance. In order to explore the pathway of cell death in the 35 chemosensitive PA1 cells, we carried out subtractive hybridization screening of a library enriched for adriamycin-induced transcripts (see Methods). Of the

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50,000 phage clones representing PA1 mRNA's expressed at 10 hrs following adriamycin treatment, 100 appeared to hybridize more strongly to a subtracted vs. control probe in primary screening. In secondary screening, 30 of the 5 initially chosen 100 plaques reproducibly displayed differential subtractive hybridization and were then used individually as probes of Northern blots carrying RNA from untreated vs. adriamycin-treated PA1 cells. $p21^{MAP1/CIP1}$ was one of six clones whose expression was increased 10 following adriamycin treatment, and its presence provided an indication that the subtraction had yielded at least one expected target. Another known gene that was isolated was *Cathepsin D*, a gene encoding a protease that was also recently isolated as an antisense suppressor of 15 cytokine-induced apoptosis. Of the other four (novel) cDNA's, one appeared to be strongly induced by adriamycin (doxorubicin) and, thus, was further characterized. Database analysis revealed that this novel gene (Figure 1) is a member of the TNF-receptor family. Based on the 20 presence of a cytoplasmic death domain (Figure 1, induction of its expression by cytotoxic chemotherapy (Figure 3) and induction of apoptosis by its overexpression, the novel gene was named *Killer*.

The *Killer* gene encodes a 411 amino acid 25 polypeptide (Figure 1) of predicted molecular weight of 45 kD. The protein contains a cytoplasmic C-terminal death domain, a transmembrane domain, and a cysteine-rich extracellular N-terminal domain characteristic of the TNF-receptor family. At the protein level, *Killer*'s 30 closest homolog is DR4 with 55% identity and an additional 9% similarity over a 454 amino acid alignment. See Figure 2A. Figure 2b shows homology between the death domain of *Killer* and other TNF-receptor family members. This alignment revealed that within the death 35 domain, *Killer* shares 64% identity with DR4, 28% with DR3, 19% with *Fas/APO1*, 29% with *TNFR-1*, and 31% with *CAR1* (Fig. 2B).

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We examined the tissue expression pattern of the Killer gene by Northern (RNA) blot analysis. Tissues screened were spleen, thymus, prostate, testis, ovary, small intestine, colon, leukocyte, heart, brain, 5 placenta, lung, liver, skeletal muscle, kidney and pancreas. A single transcript of 4.4 kb hybridized to the Killer probe and its expression appeared higher in tissues with rapid turnover as compared with nondividing tissues.

Because Killer was isolated as an adriamycin-induced transcript, and because adriamycin is a potent inducer of p53 and p53-dependent growth suppression, we explored the possibility that Killer may be a p53-regulated gene. We examined Killer mRNA expression in a series of 11 human cancer cell lines of known p53 status. Figures 3A and 3B show that Killer expression was induced following adriamycin exposure only if wild-type p53 was present but not in cells where it was mutated, degraded, or not expressed. These results strongly correlated p53-status with induction of Killer gene expression following exposure of (11/11) human cell lines to the DNA damaging Topoisomerase II inhibitor adriamycin. We further found that Killer expression could be induced by other DNA damaging agents such as ionizing radiation or etoposide (Fig. 3B for example). In order to more directly test the hypothesis that Killer is a p53-regulated gene, we infected human cancer cell lines lacking wt p53 by using a wt p53-expressing adenovirus. Fig. 3C shows that in each case tested, 25 Killer gene expression was increased by wt p53 overexpression in a manner similar to p21 (not shown). These results suggested that Killer is a gene whose expression is increased by the p53 tumor suppressor, even in the absence of DNA damage, raising the possibility that Killer may be a mediator in p53-dependent apoptosis. 30 This possibility is further supported by the fact that 35 Killer expression is elevated early (increased at 10-12

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hrs after exposure to adriamycin, gamma-radiation or Ad-p53) in cells that are undergoing apoptosis over a 24-48 hr time course.

The identification of Killer as a novel p53- and DNA damage-inducible death receptor suggested the possibility that Killer inactivation may contribute to tumor development and to resistance to apoptosis *in vivo*. In order to further explore this possibility, we determined the chromosomal localization of the Killer gene by *in-situ* hybridization using as probes three P1-clones containing the gene. Ten metaphases were examined for each P1 clone, and signal was detected on both chromosome 8's in all cells studied. The *in-situ* hybridization indicated that it localizes to chromosome 8p21, a hotspot for translocations in a number of different tumor types. Because recent experiments have demonstrated that the Type II TGF- β receptor and the *bax* genes may be mutated in colon cancers with microsatellite instability, we sequenced the death domain of Killer from normal DNA and colon cancer cell lines including 8 with microsatellite instability. We found no alteration involving a GAGAGAGA dinucleotide repeat located within the death domain of Killer (data not shown).

Because rapidly dividing tissues such as the gut express increased levels of Killer, we investigated its *in-situ* mRNA expression to determine if it correlates with apoptosis in human colon. We found that Killer is expressed in the proliferating/stem cell compartment of colonic crypt epithelia, a location which correlates with both spontaneous- and irradiation-induced apoptosis. We hypothesized that Killer expression may be downregulated in colonic tumor progression as a potential mechanism of inactivation in cancer. We found that Killer mRNA expression is low or undetectable in a colonic adenoma and two colon carcinomas with p53 mutation. In addition, the Northern blot results in Fig. 3A show that the basal expression (in the absence of adriamycin exposure) of

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Killer is lower in mutant p53-expressing SW480 cells and the p53-degrading HCT116-E6 cells as compared to wild-type p53-expressing HCT116-neo cells. It may be that loss of Killer expression in colon cancer may be due 5 to p53 mutation. In this regard, the extreme 5'-end of the Killer gene (exon 1) has been previously isolated as a methylated CpG island (Genbank accession # Z66083). DNA methyltransferase expression level has been found to be increased in colonic polyps, at a stage of 10 tumorigenesis that precedes the development of p53 mutation. It is possible that Killer gene expression may be regulated by DNA methylation, an epigenetic phenomenon that has been found to inactivate a number of tumor suppressor genes in cancer.

15 We investigated the role of Killer in apoptosis by transfecting human colon cancer cells with vectors that express the full-length Killer cDNA, its cytoplasmic domain, or the death domain. Results showed that overexpression of Killer's death domain is sufficient to 20 induce apoptosis in transfected cells. The apoptosis observed with the Killer expression vectors was similar in magnitude to p53-induced apoptosis in these cells (35% vs. 32% TUNEL-positive cells).

The death domain of Killer has been cloned into 25 a pGEX vector. Following transformation of *E. coli* cells, and induction of protein expression with IPTG, growth of bacteria was assessed over a 5 hour period. The data in Figure 4 show that expression of the death domain is also toxic to bacteria. This toxicity was not 30 a result of catabolite repression as shown by the +/- glucose controls.

We also investigated the effect of Killer on 35 growth of colon and liver cancer cells. SW480 human colon cancer cells were transfected with either pCEP4 vector or pCEP4-killer, according to protocols described above. Transfected cells were selected with 0.25 mg/ml Hygromycin for two weeks, and stained with Coomassie

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Blue. The results of this experiment clearly demonstrated that killer is a potent inhibitor of human colon cancer cell growth: cells transfected with the pCEP4-killer construct were unable to grow, while cells 5 transfected with vector alone were able to grow. Similar results were obtained with HepG2 hepatoblastoma cells.

In summary, we have isolated a novel cell death receptor (Killer) whose expression is induced by DNA damaging agents in a p53-dependent manner. Killer gene 10 expression is also inducible by wt p53 overexpression in the absence of DNA damage. Overexpression of Killer leads to cell death of cancer cells, and a number of human cancers have decreased expression of the Killer 15 gene. Members of the TNF receptor family signal apoptosis through adaptor molecules that directly activate the caspase cascade. The identification of Killer provides a potential mechanism by which DNA damaging agents can lead to p53-dependent apoptosis.

20

EXAMPLE 2

Involvement of Killer in p53-Dependent and
-Independent Apoptosis but Not Growth Arrest

Using the subtractive hybridization approach 25 described in Example 1 to isolate adriamycin-inducible transcripts from chemosensitive cells, we cloned the death receptor Killer as a DNA damage-inducible p53-regulated gene on chromosome 8p21. Overexpression of 30 Killer induces apoptosis of human cancer cells and eliminates stable colony formation. Because Killer is a novel apoptosis-inducing gene that appears to be regulated by p53, we further explore in this Example its regulation in p53-dependent G1-arrest versus apoptosis. Additionally, we examined the mechanism of regulation of 35 Killer by p53.

Materials and Methods

Cell Lines and Culture Conditions. The normal human lung fibroblast cell line WI38, the human ovarian

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cancer cell line SKOV3, the human breast cancer cell line SKBr3, and the human colon cancer cell line SW480 were obtained from the American Type Culture Collection (Rockville, MD). The human glioblastoma cell line GM (Mercer et al., Proc. Natl. Acad. Sci. USA 87: 6166-6170, 5 1990) was obtained from Ed Mercer (Thomas Jefferson University).

Adenovirus Infection. The wild-type p53-expressing replication deficient adenovirus Ad-p53 and the LacZ-expressing adenovirus Ad-LacZ (El Deiry et 10 al., 1993, *supra*) were obtained from Bert Vogelstein (Johns Hopkins University). Titering and infection with adenoviruses was carried out at an MOI of 50 as previously described (El Deiry et al., 1993, *supra*; 15 Blagoskonny & El Deiry, 1996, *supra*).

RNA extraction and Northern Blot. RNA extraction and Northern blot were as described in Example 1. The full-length (1.34 kb) human Killer cDNA probe was described in Example 1. The full-length human 20 p21WAF1/CIP1, MDM2 and p53 cDNA's are described elsewhere (El Deiry et al., 1993, *supra*). A human bax probe (Oltvai et al., Cell 74: 609-616, 1993; Miyashita et al., Oncogene 9: 1799-1805, 1994; Miyashita et al., Cell 80: 293-299, 1995) was provided by John Reed (Burnham 25 Institute).

Apoptosis assays. GM cells were infected by Ad-LacZ or Ad-p53. After 30 hrs, cells were stained with DAPI as previously described (Blagoskonny & El Deiry, 1996, *supra*).

Determination of messenger RNA stability. 30 SW480 human colon adenocarcinoma cells were infected by either Ad-LacZ or Ad-p53 for one hour using an MOI of 50 as previously described (Blagosklonny & El Deiry, 1996, *supra*; Meng et al, Clin. Cancer Res. 4: 251-259, 1998). 35 After an additional incubation at 37°C for 14 hrs., Actinomycin D was added to the cultures at a final concentration of 30 ng/ml. Total RNA was harvested at

- 40 -

different time points after Actinomycin D addition as indicated in Fig. 4, and Northern blotting was performed as described above.

5 Results

Killer expression is not increased in p53-dependent growth arrest. One of the major questions in elucidating p53 function has been to understand what determines whether cells undergo cell cycle arrest or apoptosis following p53 activation. We have found a perfect correlation between induction of Killer expression and exposure of wild-type p53-expressing human cancer cells to apoptosis-inducing cytotoxic agents (Example 1). Following exposure to lethal doses of agents such as adriamycin, etoposide, or ionizing radiation, only cells which contain wild-type p53 induced Killer expression. Cells with mutant or degraded p53 failed to upregulate Killer expression in response to genotoxic drugs. In order to investigate whether the p53-dependent upregulation of Killer is specific to cells undergoing apoptosis, we used two well established systems where p53 induction leads only to cell cycle arrest.

The human glioblastoma cell line GM contains an endogenous mutant p53 and an exogenous dexamethasone-inducible wild-type p53. Upon addition of dexamethasone to the media, it has been shown that these cells induce expression of wild-type p53 which in turn transcriptionally activates expression of p21, and the cells then undergo cell cycle arrest in G1-phase and not apoptosis. Fig. 5A shows that upon addition of dexamethasone, the expected induction in p21 expression is observed, but there is no accompanying upregulation of Killer expression. These results suggest that cells undergoing p53-dependent cell cycle arrest induce p21 as expected but do not induce expression of Killer, a putative mediator of p53-dependent apoptosis.

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In order to validate these observations in a different system, we chose to examine the expression levels of p21 and Killer in WI38 normal human lung fibroblast cells either with no treatment or following exposure to ionizing radiation. It has been shown previously that the wild-type p53 containing WI38 lung fibroblasts respond to g- irradiation by undergoing a prolonged cell cycle arrest associated with p21 upregulation, and not apoptosis. Following irradiation, p21 expression was induced as expected but Killer expression was not increased (Fig. 5B). In fact at 10 Gy, Killer mRNA expression appeared to be inhibited. These results are consistent with the hypothesis that the p53-dependent induction of Killer is "apoptosis-specific" and not a general response in cells undergoing only p53-dependent cell cycle arrest.

Increased expression of Killer in cells undergoing p53-dependent apoptosis. To further investigate the hypothesis that p53-dependent upregulation of Killer expression may be a determinant of apoptosis induction, we infected GM cells with Ad-p53. GM cells were found to undergo apoptosis following Ad-p53 as compared to Ad-LacZ infection or dexamethasone treatment (Fig. 5A). Unlike the dexamethasone-treated GM cells which do not undergo p53-dependent apoptosis, the Ad-p53 infected cells upregulated expression of the Killer gene. We suspect that the higher levels of p53 achieved following Ad-p53 infection probably contribute to the apoptosis phenotype in GM cells. These results further correlate the p53-dependent induction of Killer with apoptosis.

We further investigated the kinetics of Killer upregulated expression following Ad-p53 as compared to Ad-LacZ infection of the SKOV3 human ovarian carcinoma cell line (Fig. 6B). Expression of Killer was detectably increased as early as 6 hours following Ad-p53 infection of the SKOV3 cells. This pattern of expression would be

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expected for genes involved in p53 mediated apoptosis which occurs over 1-3 days following Ad-p53 infection (Fig. 6A and data not shown).

Transcriptional and post-transcriptional control of TRAIL death receptor expression in p53-dependent apoptosis. We further explored the mechanism by which Killer is induced following p53 overexpression in cancer cells undergoing apoptosis. We infected SKOV3 cells (Fig. 7A) or GM cells (Fig. 7B) using either Ad-LacZ or Ad-p53 for one hour followed by a 20 hr incubation in the presence of the transcription inhibitor Actinomycin D. Expression of the p53 targets p21, MDM2 and Killer was greatly increased in Ad-p53 as compared with Ad-LacZ infected SKOV3 cells in the absence of Actinomycin D (Fig. 7A, compare lanes 3 to lanes 1). In the presence of Actinomycin D, the p53-dependent upregulation of p21, MDM2, and Killer was abrogated (Fig. 7A, compare lanes 4 to lanes 2). These results suggest that Killer, p21 and MDM2 appear to be regulated by p53 at the level of transcription. p53 mRNA levels were also not decreased by Actinomycin D in Ad-p53 infected cells (Fig 7A).

We investigated the effect of Actinomycin D on the expression of Killer following infection of GM cells by Ad-p53 or Ad-LacZ (Fig. 3B). The p53- dependent upregulation of Killer expression (Fig. 7B lane 3 vs. 1, upper panel) was blocked by Actinomycin D in GM cells (lane 4 vs. 3).

The present invention is not limited to the embodiments specifically described above, but is capable of variation and modification without departure from the scope of the appended claims.

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

5 (i) APPLICANT: Trustees of the University of Pennsylvania
(ii) TITLE OF THE INVENTION: NUCLEIC ACID ENCODING A NOVEL
CHEMOTHERAPY-INDUCED PROTEIN, AND METHODS OF USE

10 (iii) NUMBER OF SEQUENCES: 13

(iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE: Dann, Dorfman, Herrell & Skillman, P.C.
(B) STREET: 1601 Market Street, Suite 720
(C) CITY: Philadelphia
(D) STATE: Pennsylvania
(E) COUNTRY: USA
(F) ZIP: 19103

20 COMPUTER READABLE FORM:

(v) COMPUTER READABLE DEVICE
(A) MEDIUM TYPE: Diskette
(B) COMPUTER: IBM Compatible
(C) OPERATING SYSTEM: DOS
(D) SOFTWARE: FastSEQ Version 1

25 (D) CONTINUED

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER: Not yet assigned

(B) FILING DATE: 10-JUL-1998

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 (A) APPLICATION NUMBER: US 60/052,305
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(B) FILING DATE: 30-SEP-1997

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Janet E. Reed, Ph.D.
(B) REGISTRATION NUMBER: 36,252
(C) REFERENCE/DOCKET NUMBER: PENN J1638PCT

(ix) TELECOMMUNICATION INFORMATION:
 (A) TELEPHONE: 215-563-4100
 (B) TELEFAX: 215-563-4044
 (C) TELEX:

(2) INFORMATION FOR SEQ ID NO:1:

55 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 1139 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

60 (ii) MOLECULE TYPE: cDNA
(iii) HYPOTHETICAL: NO

(iv) ANTISENSE: NO
(v) FRAGMENT TYPE:
(vi) ORIGINAL SOURCE: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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	ATGGAACAAAC	GGGGACAGAA	CGCCCCGGCC	GCTTCGGGG	CCCGGAAAAG	CCACGGCCCA	60
	GGACCAGGG	AGGGCGGGG	AGCCAGGCCT	GGGCCCGGG	TCCCAAGAC	CCTTGTGCTC	120
	GTTGTCGCG	CGGTCTGCT	GTTGGTCTA	GCTGAGTCTG	CTCTGATCAC	CCAACAAGAC	180
	CTAGCTCCC	AGCAGAGAGC	GGCCCCAACAA	CAAAAGAGGT	CCAGCCCCCTC	AGAGGGATTG	240
5	TGTCCACCTG	GACACCATAT	CTCAGAAGAC	GGTAGAGATT	GCATCTCCCT	CAAATATGGA	300
	CAGGACTATA	GCACTCACTG	GAATGACCTC	CTTTTCTGCT	TGCGCTGCAC	CAGGTGTGAT	360
	TCAGGTGAAG	TGGAGCTAAG	TCCCTGCACC	ACGACAGGAA	ACACAGTGTG	TCAGTGCAG	420
	AAGGCACCTT	CCGGGAAGAA	GATTCTCCCTG	AGATGTCGCC	GAAGTGCAGC	ACAGGGTGTG	480
	CCAGAGGGAT	GGTCAAGGTC	GGTAGATTGTA	ACACCTGGAG	TGACATCGAA	TGTGTCCACA	540
10	AAGAATCAGG	CATCATCATA	GGAGTCACAG	TTGCAGCCGT	AGTCTTGATT	GTGGCTGTGT	600
	TTGTTTCAA	GTCTTACTG	TGGAAGAAAG	TCCTCCCTTA	CCTGAAAGGC	ATCTGCTCAG	660
	GTGGTGGTGG	GGACCCCTGAG	CGTGTGGACA	GAAGCTACA	ACGACCTGGG	GCTGAGGACAA	720
	ATGTCCTCAA	TGAGATCGTG	AGTATCTTGC	AGCCCACCCA	GGTCCCTGAG	CAGGAAATGG	780
	AAGTCCAGGA	GCCAGCAGAG	CCAACAGGTG	TCAACATGTT	GTCCCCCGGG	GAGTCAGAGC	840
15	ATCTGCTGGA	ACCGGCAGAA	GCTGAAAGGT	CTCAGAGGAG	GAGGCTGCTG	GTTCCAGCAA	900
	ATGAAGGTGA	TCCCACTGAG	ACTCTGAGAC	AGTCTTCGA	TGACTTTGCA	GACTTGGTGC	960
	CCTTTGACTC	CTGGGAGCCG	CTCATGAGGA	AGTTGGCCT	CATGGACAAAT	GAGATAAAGG	1020
	TGGCTAAAGC	TGAGGGCAGCG	GGCCACAGGG	ACACCTGTA	CACGATGCTG	ATAAAGTGGG	1080
	TCAACAAAAC	CGGGCGAGAT	GCCTCTGTCC	ACACCTGCT	GGATGCCTG	GAGACGCTG	1139

20 (2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 411 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTISENSE: NO

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE: *Homo sapiens*

35 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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	1				5					10					15	
40	Arg	His	Gly	Pro	Gly	Pro	Arg	Glu	Ala	Arg	Gly	Ala	Arg	Pro	Gly	Pro
							20			25					30	
	Arg	Val	Pro	Lys	Thr	Leu	Val	Leu	Val	Val	Ala	Ala	Val	Leu	Leu	Leu
								35		40				45		
	Val	Ser	Ala	Glu	Ser	Ala	Leu	Ile	Thr	Gln	Gln	Asp	Leu	Ala	Pro	Gln
45									55				60			
	Gln	Arg	Ala	Ala	Pro	Gln	Gln	Lys	Arg	Ser	Ser	Pro	Ser	Glu	Gly	Leu
	65						70				75			80		
	Cys	Pro	Pro	Gly	His	His	Ile	Ser	Glu	Asp	Gly	Arg	Asp	Cys	Ile	Ser
							85			90				95		
50	Cys	Lys	Tyr	Gly	Gln	Asp	Tyr	Ser	Thr	His	Trp	Asn	Asp	Leu	Leu	Phe
						100			105				110			
	Cys	Leu	Arg	Cys	Thr	Arg	Cys	Asp	Ser	Gly	Glu	Val	Glu	Leu	Ser	Pro
						115			120				125			
	Cys	Thr	Thr	Thr	Arg	Asn	Thr	Val	Cys	Gln	Cys	Glu	Glu	Gly	Thr	Phe
						130			135				140			
55	Arg	Glu	Glu	Asp	Ser	Pro	Glu	Met	Cys	Arg	Lys	Cys	Arg	Thr	Gly	Cys
	145						150				155			160		
	Pro	Arg	Gly	Met	Val	Lys	Val	Gly	Asp	Cys	Thr	Pro	Trp	Ser	Asp	Ile
							165			170				175		
60	Glu	Cys	Val	His	Lys	Glu	Ser	Gly	Ile	Ile	Ile	Gly	Val	Thr	Val	Ala
						180				185				190		
	Ala	Val	Val	Leu	Ile	Val	Ala	Val	Phe	Val	Cys	Lys	Ser	Leu	Leu	Trp
						195				200				205		
	Lys	Lys	Val	Leu	Pro	Tyr	Leu	Lys	Gly	Ile	Cys	Ser	Gly	Gly	Gly	Gly
65	Asp	Pro	Glu	Arg	Val	Asp	Arg	Ser	Ser	Gln	Arg	Pro	Gly	Ala	Glu	Asp
	225						230				235			240		
	Asn	Val	Leu	Asn	Glu	Ile	Val	Ser	Ile	Leu	Gln	Pro	Thr	Gln	Val	Pro

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	245	250	255
	Glu Gln Glu Met	Glu Val Gln Glu Pro Ala Glu Pro Thr Gly	Val Asn
	260	265	270
5	Met Leu Ser Pro Gly Glu Ser Glu His Leu Leu Glu Pro Ala Glu Ala		
	275	280	285
	Glu Arg Ser Gln Arg Arg Arg	Leu Leu Val Pro Ala Asn Glu Gly Asp	
	290	295	300
	Pro Thr Glu Thr Leu Arg Gln Cys Phe Asp Asp	Phe Ala Asp Leu Val	
10	305	310	315
	Pro Phe Asp Ser Trp Glu Pro Leu Met Arg Lys Leu Gly Leu Met Asp		
	325	330	335
	Asn Glu Ile Lys Val Ala Lys Ala Glu Ala Ala Gly His Arg Asp Thr		
	340	345	350
15	Leu Tyr Thr Met Leu Ile Lys Trp Val Asn Lys Thr Gly Arg Asp Ala		
	355	360	365
	Ser Val His Thr Leu Leu Asp Ala Leu Glu Thr Leu Gly Glu Arg Leu		
	370	375	380
	Ala Lys Gln Lys Ile Glu Asp His Leu Leu Ser Ser Gly Lys Phe Met		
	385	390	395
20	Tyr Leu Glu Gly Asn Ala Asp Ser Ala Met Ser		
	405	410	400

(2) INFORMATION FOR SEQ ID NO:3:

25 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 468 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

30 (ii) MOLECULE TYPE: peptide
 (iii) HYPOTHETICAL: NO
 (iv) ANTISENSE: NO
 (v) FRAGMENT TYPE: internal
 (vi) ORIGINAL SOURCE: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

40	Met Ala Pro Pro Pro Ala Arg Val His Leu Gly Ala Phe Leu Ala Val 1 5 10 15 Thr Pro Asn Pro Gly Ser Ala Ala Ser Gly Thr Glu Ala Ala Ala Ala 20 25 30 Thr Pro Ser Lys Val Trp Gly Ser Ser Ala Gly Arg Ile Glu Pro Arg 35 40 45 45 Gly Gly Arg Gly Ala Leu Pro Thr Ser Met Gly Gln His Gly Pro 50 55 60 Ser Ala Arg Ala Arg Ala Gly Arg Ala Pro Gly Pro Arg Pro Ala Arg 65 70 75 80 50 Glu Ala Ser Pro Arg Leu Arg Val His Lys Thr Phe Lys Phe Val Val 85 90 95 Val Gly Val Leu Leu Gln Val Val Pro Ser Ser Ala Ala Thr Ile Lys 100 105 110 Leu His Asp Gln Ser Ile Gly Thr Gln Gln Trp Glu His Ser Pro Leu 115 120 125 55 Gly Glu Leu Cys Pro Pro Gly Ser His Arg Ser Glu Arg Pro Gly Ala 130 135 140 Cys Asn Arg Cys Thr Glu Gly Val Gly Tyr Thr Asn Ala Ser Asn Asn 145 150 155 160 Leu Phe Ala Cys Leu Pro Cys Thr Ala Cys Lys Ser Asp Glu Glu 165 170 175 60 Arg Ser Pro Cys Thr Thr Arg Asn Thr Ala Cys Gln Cys Lys Pro 180 185 190 Gly Thr Phe Arg Asn Asp Asn Ser Ala Glu Met Cys Arg Lys Cys Ser 195 200 205 65 Thr Gly Cys Pro Arg Gly Met Val Lys Val Lys Asp Cys Thr Pro Trp 210 215 220 Ser Asp Ile Glu Cys Val His Lys Glu Ser Gly Asn Gly His Asn Ile
----	--

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		230		235		240
225	Trp Val Ile Leu Val Val Thr Leu Val Val Pro Leu Leu Leu Val Ala					
	245			250		255
5	Val Leu Ile Val Cys Cys Cys Ile Gly Ser Gly Cys Gly Gly Asp Pro					
	260		265			270
	Lys Cys Met Asp Arg Val Cys Phe Trp Arg Leu Gly Leu Leu Arg Gly					
	275		280		285	
10	Pro Gly Ala Glu Asp Asn Ala His Asn Glu Ile Leu Ser Asn Ala Asp					
	290		295		300	
	Ser Leu Ser Thr Phe Val Ser Glu Gln Gln Met Glu Ser Gln Glu Pro					
	305		310		315	
	Ala Asp Leu Thr Gly Val Thr Val Gln Ser Pro Gly Glu Ala Gln Cys					
	325		330			335
15	Leu Leu Gly Pro Ala Glu Ala Glu Gly Ser Gln Arg Arg Arg Leu Leu					
	340		345			350
	Val Pro Ala Asn Gly Ala Asp Pro Thr Glu Thr Leu Met Leu Phe Phe					
	355		360		365	
	Asp Lys Phe Ala Asn Ile Val Pro Phe Asp Ser Trp Asp Gln Leu Met					
	370		375		380	
20	Arg Gln Leu Asp Leu Thr Lys Asn Glu Ile Asp Val Val Arg Ala Gly					
	385		390		395	
	Thr Ala Gly Pro Gly Asp Ala Leu Tyr Ala Met Leu Met Lys Trp Val					
	405		410			415
25	Asn Lys Thr Gly Arg Asn Ala Ser Ile His Thr Leu Leu Asp Ala Leu					
	420		425			430
	Glu Arg Met Glu Glu Arg His Ala Lys Glu Lys Ile Gln Asp Leu Leu					
	435		440		445	
	Val Asp Ser Gly Lys Phe Ile Tyr Leu Glu Asp Gly Thr Gly Ser Ala					
	450		455		460	
30	Val Ser Leu Glu					
	465					

(2) INFORMATION FOR SEQ ID NO:4:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Arg Trp Lys Glu Phe Val Arg Thr Leu Gly Leu Arg Glu Ala Glu Ile
 50 1 5 10 15
 Glu Ala Val Glu Val Glu Ile Gly Arg Phe Arg Asp Gln Gln Tyr Glu
 20 25 30
 Met Leu Lys Arg Trp Arg Gln Gln Pro Ala Gly Leu Gly Ala Val
 35 40 45
 55 50 55 60
 Tyr Ala Ala Leu Glu Arg Met Gly Leu Asp Gly Cys Val Glu Asp Leu
 Arg
 65

60 (2) INFORMATION FOR SEQ ID NO:5:

65 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 68 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

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- (ii) MOLECULE TYPE: peptide
- (iii) HYPOTHETICAL: NO
- (iv) ANTISENSE: NO
- (v) FRAGMENT TYPE: internal
- (vi) ORIGINAL SOURCE: Homo sapiens

5 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

20 (2) INFORMATION FOR SEQ ID NO:6:

25 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 69 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

30 (ii) MOLECULE TYPE: peptide
 (iii) HYPOTHETICAL: NO
 (iv) ANTISENSE: NO
 (v) FRAGMENT TYPE: internal
 (vi) ORIGINAL SOURCE: homo sapiens

(2) INFORMATION FOR SEQ ID NO:7:

50 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 67 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

55 (ii) MOLECULE TYPE: peptide
 (iii) HYPOTHETICAL: NO
 (iv) ANTISENSE: NO
 (v) FRAGMENT TYPE: internal
 (vi) ORIGINAL SOURCE: Homo sapiens

60 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

(11) 1
 Glu Trp Lys Arg Phe Gly Arg Ala Leu Asp Leu Gln Glu Asn Asp Leu
 1 5 10 15
 Tyr Leu Ala Glu Gln His Glu Arg Val Ser Cys Glu Pro Arg Tyr Gln
 20 25 30
 Met Leu Asn Thr Trp Leu Asn Gln Gln Gly Ser Lys Ala Ser Val Asn

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- 48 -

35 40 45
 Thr Leu Leu Glu Thr Leu Pro Arg Ile Gly Leu Ser Gly Val Ala Asp
 50 55 60
 Ile Ile Ala
 5 65

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: cDNA
- (iii) HYPOTHETICAL: NO
- (iv) ANTISENSE: NO
- (v) FRAGMENT TYPE:
- (vi) ORIGINAL SOURCE: synthetic

20 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

CCCCAAGCTTC CGCCATGGAA CAACGGGGAC

30

25 (2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: cDNA
- (iii) HYPOTHETICAL: NO
- (iv) ANTISENSE: NO
- (v) FRAGMENT TYPE:
- (vi) ORIGINAL SOURCE: synthetic

35 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

40 CGCGGATCCT TAGGACATGG CAGAGTCTGC

30

(2) INFORMATION FOR SEQ ID NO:10:

45 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 29 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: cDNA
- (iii) HYPOTHETICAL: NO
- (iv) ANTISENSE: NO
- (v) FRAGMENT TYPE:

- (vi) ORIGINAL SOURCE: synthetic

55 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

CGCGGATCCG TCCTCAATCT TCTGCTTGG

29

60 (2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 35 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

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(iii) MOLECULE TYPE: cDNA
(iii) HYPOTHETICAL: NO
(iv) ANTISENSE: NO
(v) FRAGMENT TYPE:
5 (vi) ORIGINAL SOURCE: synthetic
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

CCCAAGCTTC CACCATGGTT GCAGCCGTAG TCTTG

35

10 (2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
15 (B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA
20 (iii) HYPOTHETICAL: NO
(iv) ANTISENSE: NO
(v) FRAGMENT TYPE:
(vi) ORIGINAL SOURCE: synthetic

25 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

CCCAAGCTTC CACCATGGAC TCCTGGGAGC CGC

33

30 (2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 29 base pairs
(B) TYPE: nucleic acid
35 (C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA
(iii) HYPOTHETICAL: NO
(iv) ANTISENSE: NO
40 (v) FRAGMENT TYPE:
(vi) ORIGINAL SOURCE: synthetic

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

45 CGCGGATCCG TCCTCAATCT TCTGCTTGG

29

- 50 -

What is claimed is:

1. An isolated nucleic acid molecule comprising an open reading frame of part or all of a gene located on human chromosome 8p21, which encodes part or 5 all of a mammalian p53-inducible, apoptosis-mediating protein, which comprises an amino-terminal extracellular receptor domain, a transmembrane domain, and a death domain for protein-protein interactions.
- 10 2. The nucleic acid molecule of claim 1, which encodes a human protein.
- 15 3. The nucleic acid molecule of claim 2, which is DNA.
- 20 4. The DNA molecule of claim 3, which is a cDNA comprising a sequence approximately 1.2 kilobase pairs in length that encodes said p53-inducible, apoptosis-mediating protein.
- 25 5. The DNA molecule of claim 3, which is a gene, the exons of which comprise said open reading frame.
- 30 6. The nucleic acid molecule of claim 2, which encodes a polypeptide having a sequence substantially the same as SEQ ID NO:2.
- 35 7. The nucleic acid molecule of claim 6, which encodes SEQ ID NO:2.
8. The nucleic acid molecule of claim 8, which comprises SEQ ID NO:1.
- 35 9. A vector for transformation of a mammalian cell, which comprises the nucleic acid molecule of claim 1.

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10. A mammalian cell transformed with the vector of claim 9.

11. An oligonucleotide between 10 and 100 5 nucleotides in length, that specifically hybridizes with a pre-determined portion of the nucleic acid molecule of claim 1.

12. An isolated nucleic acid molecule having a 10 sequence selected from the group consisting of:
a) SEQ ID NO:1;
b) an allelic variant or natural mutant of SEQ ID NO:1;
c) a sequence hybridizing with part 15 or all of SEQ ID NO:1 or its complement and encoding a polypeptide substantially the same as part or all of a polypeptide encoded by SEQ ID NO:1; and
d) a sequence encoding part or all of a polypeptide having amino acid SEQ ID NO:2.

20 13. A protein produced by expression of an isolated nucleic acid molecule comprising part or all of an open reading frame of a gene located on human chromosome 8p21, said protein being a p53-induced 25 apoptosis-mediating protein having an amino-terminal extracellular receptor domain, a transmembrane domain, and a death domain for protein-protein interactions.

30 14. The protein of claim 13, comprising an amino acid sequence substantially the same as SEQ ID NO:2.

35 15. The protein of claim 13, produced by expression of an open reading frame having a sequence substantially the same as SEQ ID NO:1.

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16. Antibodies immunologically specific for
the protein of claim 13.

17. A protein produced by expression of a
sequence selected from the group consisting of:
5 a) SEQ ID NO:1;
b) an allelic variant or natural
mutant of SEQ ID NO:1;
c) a sequence hybridizing with part
10 or all of SEQ ID NO:1 or its complement and encoding a
polypeptide substantially the same as part or all of a
polypeptide encoded by SEQ ID NO:1; and
d) a sequence encoding part or all
of a polypeptide having amino acid SEQ ID NO:2.

15 18. Antibodies immunologically specific for
the protein of claim 17.

19. A non-human genetically engineered mammal,
20 which is unable to produce a functional protein normally
produced by expression of an isolated nucleic acid
molecule comprising part or all of an open reading frame
of a gene located on human chromosome 8p21, said protein
being a p53-induced apoptosis-mediating protein having an
25 amino-terminal extracellular receptor domain, a
transmembrane domain, and a death domain for protein-
protein interactions.

20. The genetically engineered mammal of claim
30 19, which is a mouse.

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FIG. 1

Killer protein	1	QNAPAASGARKKRHGPGPAREARGARGPGRVPKTLVLUVAVL	48
DR4 protein	1	ASGTEAAATPSKVWGGSSAGRIEPRGGGRGA LPPTSMGQHGPSAR-ARAGRAPGPPAREASPRLRVHKTFKVVVGVLLQ	79
Killer protein	49	MEQRG-----QNAPAASGARKKRHGPGPAREARGARGPGRVPKTLVLUVAVL	48
DR4 protein	80	VVPSSAATIKLHDQS---IGTQQWEHSPLGECLCPPSHRSERPGACNRCTEGVGTNASNNLFACLPCTACKSDEEERSP	156
Killer protein	129	VSAESALITQQQLAPQORAAPQQKRSSEGLCPGPGHKISEDGRDC1ISCKYQGDYSTHWNDLJFCLRRRTRCDSGEVELSP	128
DR4 protein	157	VSAESALITQQQLAPQORAAPQQKRSSEGLCPGPGHKISEDGRDC1ISCKYQGDYSTHWNDLJFCLRRRTRCDSGEVELSP	128
Killer protein	209	CTTTRNTVCQCEEGTFREEDSPEMCRKCRTGCPGMVKVGDCTPWSDIECVHKESGIIIGVTVAAVVLIVAVFUCKSLLW	208
DR4 protein	232	CTTTRNTVCQCEEGTFREEDSPEMCRKCRTGCPGMVKVGDCTPWSDIECVHKESGIIIGVTVAAVVLIVAVFUCKSLLW	208
Killer protein	280	CTTTRNTACQCKPGTFRNDNSAEMCRKCSSTGGCPGMVKVGDCTPWSDIECVHKESGIIIGVTVAAVVLIVAVFUCKSLLW	208
DR4 protein	312	CTTTRNTACQCKPGTFRNDNSAEMCRKCSSTGGCPGMVKVGDCTPWSDIECVHKESGIIIGVTVAAVVLIVAVFUCKSLLW	208
Killer protein	209	CTTTRNTACQCKPGTFRNDNSAEMCRKCSSTGGCPGMVKVGDCTPWSDIECVHKESGIIIGVTVAAVVLIVAVFUCKSLLW	208
DR4 protein	232	CTTTRNTACQCKPGTFRNDNSAEMCRKCSSTGGCPGMVKVGDCTPWSDIECVHKESGIIIGVTVAAVVLIVAVFUCKSLLW	208
Killer protein	280	KKVLPYLKICSGGGGGDPERVDRSSQR-----PGAEDNVLEIVS--ILQPTQVPEQEMEVQEPAEPGTGVNMILSPGES	279
DR4 protein	312	KKVLPYLKICSGGGGGDPERVDRSSQR-----PGAEDNVLEIVS--ILQPTQVPEQEMEVQEPAEPGTGVNMILSPGES	279
Killer protein	209	KKVLPYLKICSGGGGGDPERVDRSSQR-----PGAEDNVLEIVS--ILQPTQVPEQEMEVQEPAEPGTGVNMILSPGES	279
DR4 protein	232	KKVLPYLKICSGGGGGDPERVDRSSQR-----PGAEDNVLEIVS--ILQPTQVPEQEMEVQEPAEPGTGVNMILSPGES	279
Killer protein	280	RKVLVYCCIGSGCGGGDPKCMDRVCFWRLGLRGPGGAEDNAHNEILSNADSLSTFVSEQQMESESQEPADLTGTVTQSPGEA	311
DR4 protein	312	RKVLVYCCIGSGCGGGDPKCMDRVCFWRLGLRGPGGAEDNAHNEILSNADSLSTFVSEQQMESESQEPADLTGTVTQSPGEA	311
Killer protein	280	EHLLEPAAERSQRRLLVLPANEQGDPTETLRCQCFDDFAIDLVPFDWEPLMRKLGMDNEIKVAKAEAAGHRDTLYTMLIK	359
DR4 protein	312	EHLLEPAAERSQRRLLVLPANEQGDPTETLRCQCFDDFAIDLVPFDWEPLMRKLGMDNEIKVAKAEAAGHRDTLYTMLIK	359
Killer protein	280	QCLLGPAAEGSQRRLLVLPANGADPTETLMLFFDKFANTIVPDFDSWDLQMRQLDLTKEIDVVRAGTAGPGDALYAMLMK	391
DR4 protein	312	QCLLGPAAEGSQRRLLVLPANGADPTETLMLFFDKFANTIVPDFDSWDLQMRQLDLTKEIDVVRAGTAGPGDALYAMLMK	391
Killer protein	360	WVAKTGRDASVHTLIDDALETLGERLAQKIEDHLLSSGKFMLEGNADSAMS	411
DR4 protein	392	WVAKTGRNASIHTLIDDALETLGERLAQKIEDHLLSSGKFMLEGNADSAMS	445

FIG. 2A

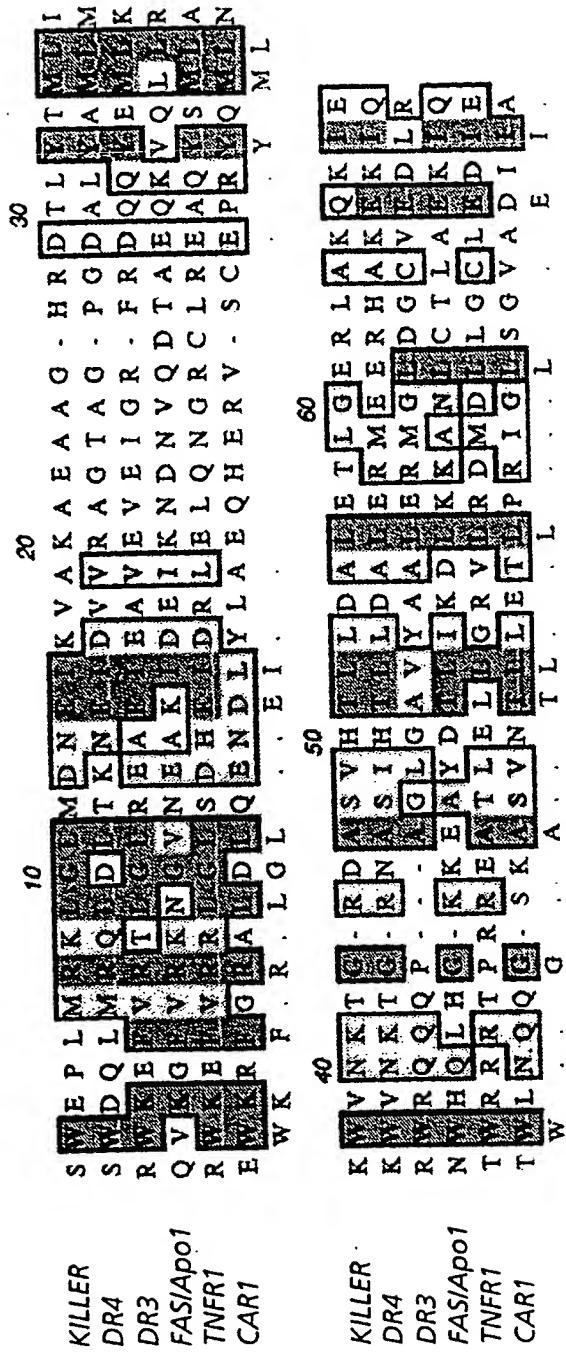
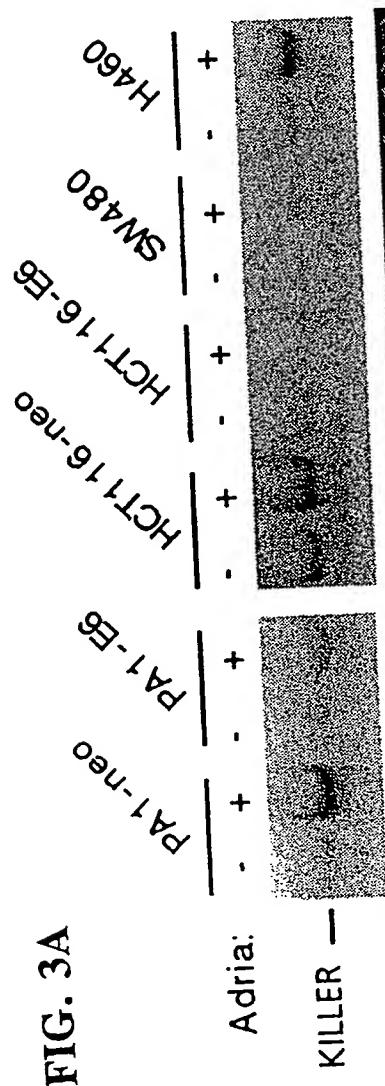
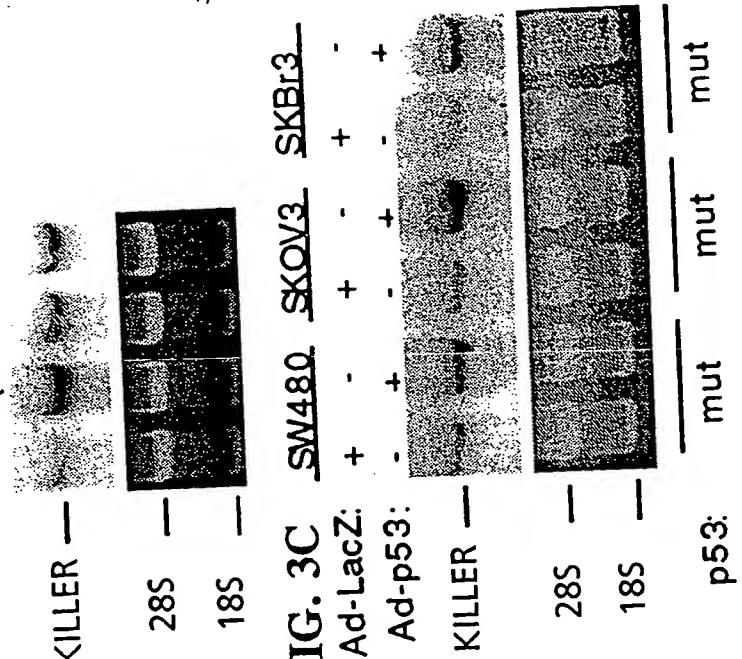
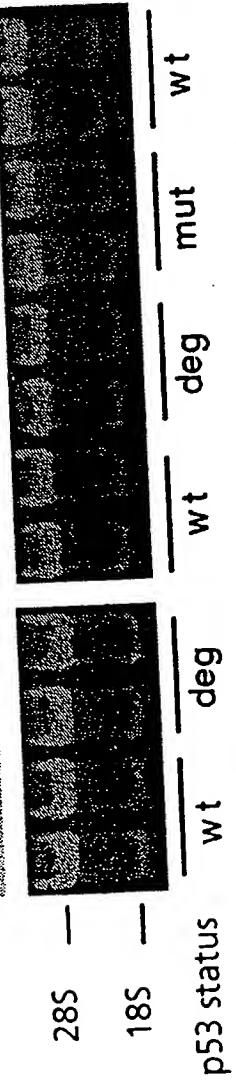


FIG. 2B

FIG. 3A**FIG. 3B**
Untreated Adriamycin
 γ -irradiation**FIG. 3C**

p53: mut — mut — mut

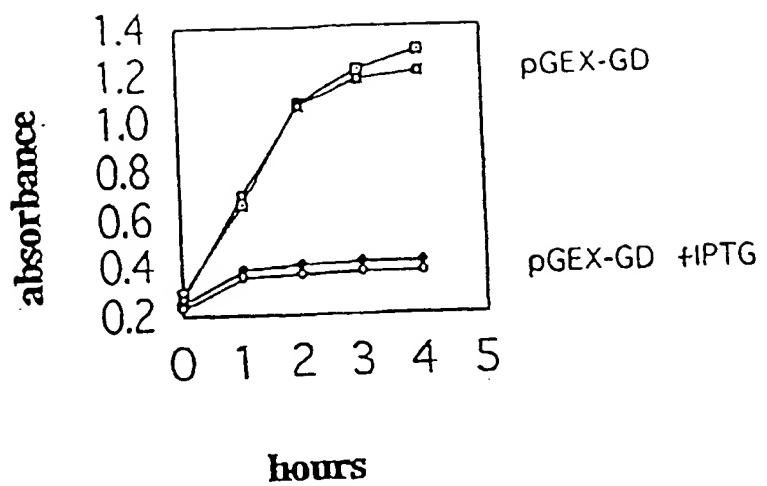


FIG. 4

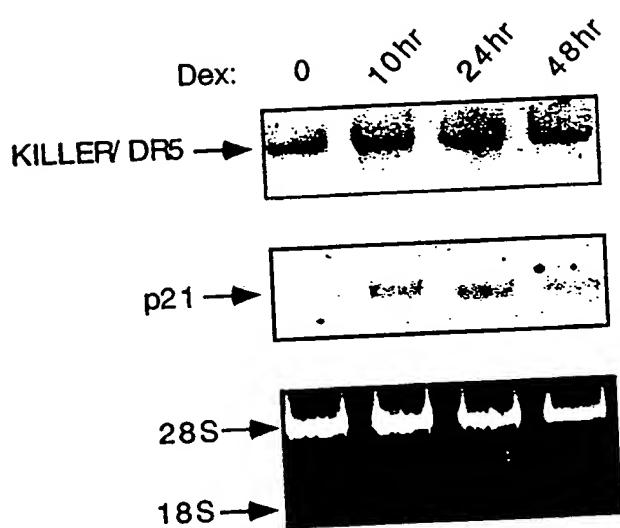
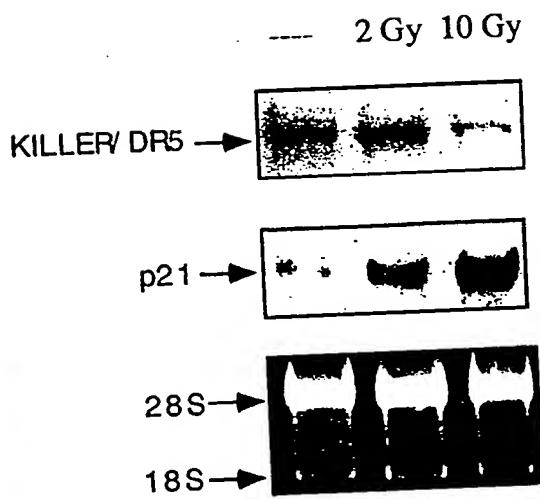
FIG. 5A**GM Cells****FIG. 5B****WI38 Cells**

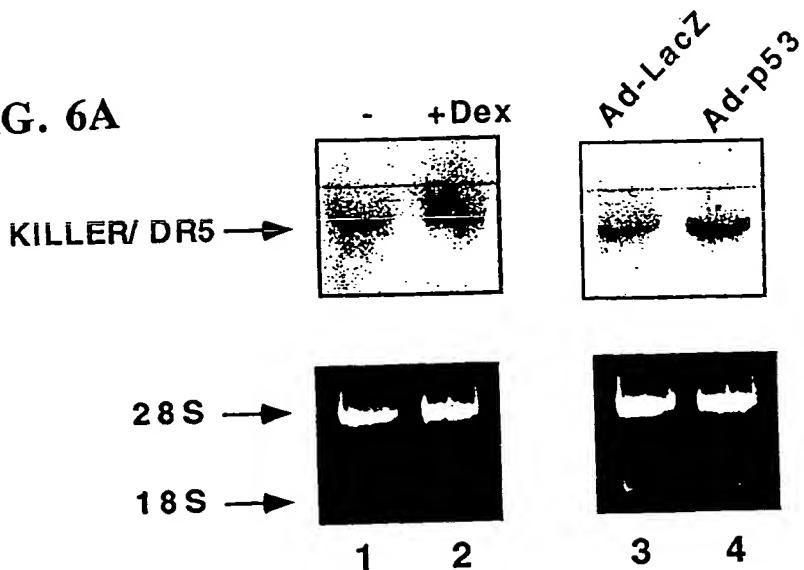
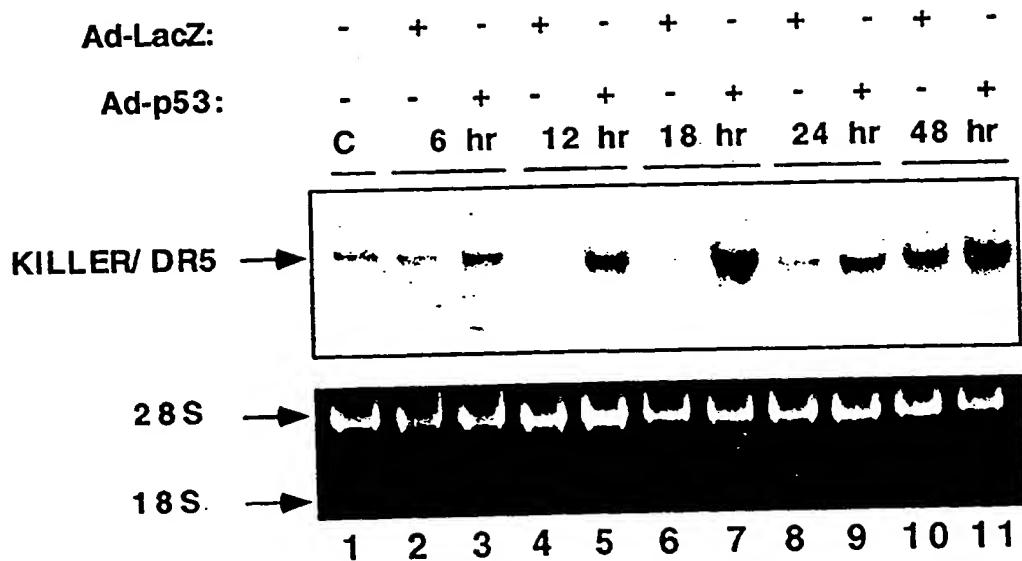
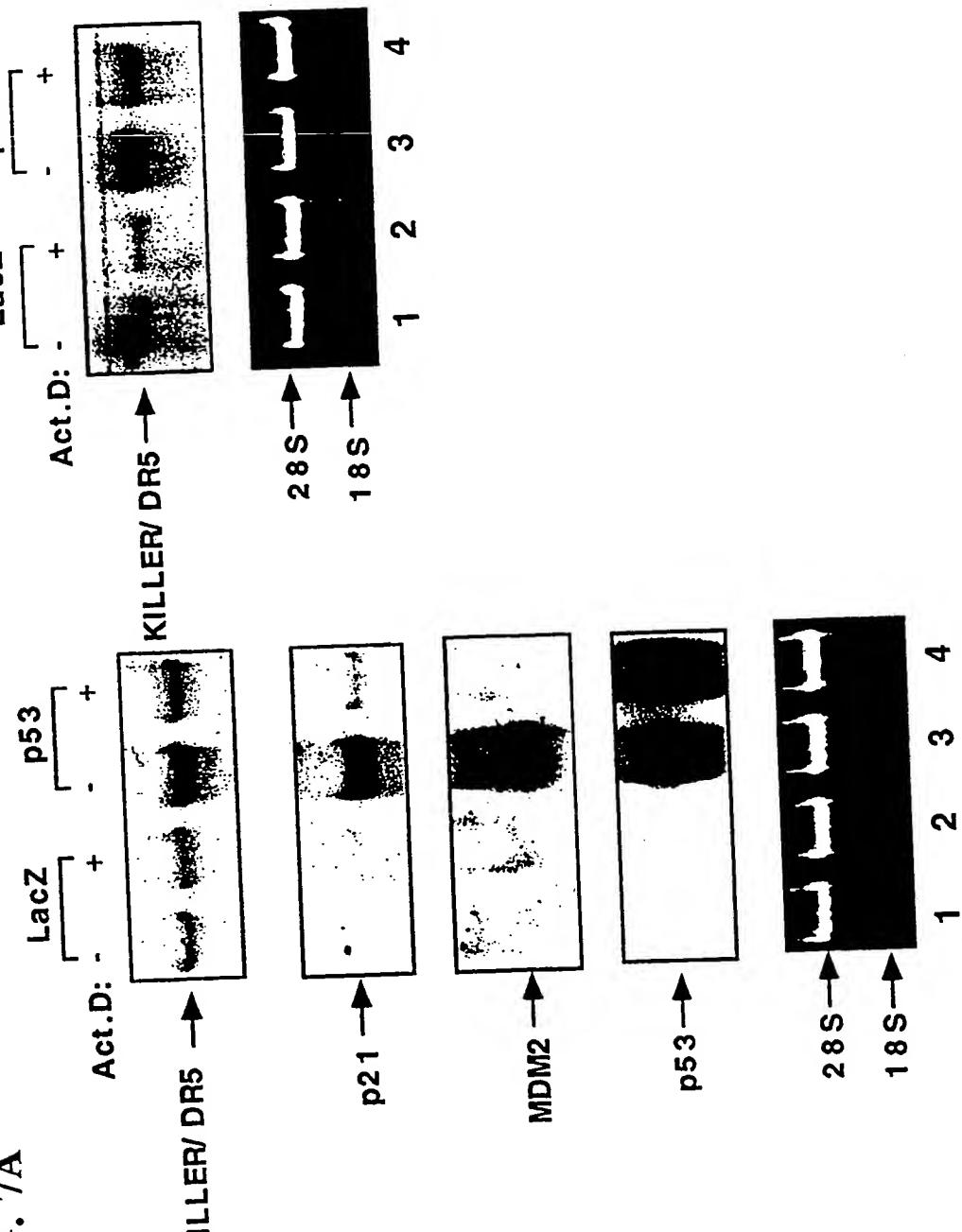
FIG. 6A**FIG. 6B**

FIG. 7A

FIG. 7B



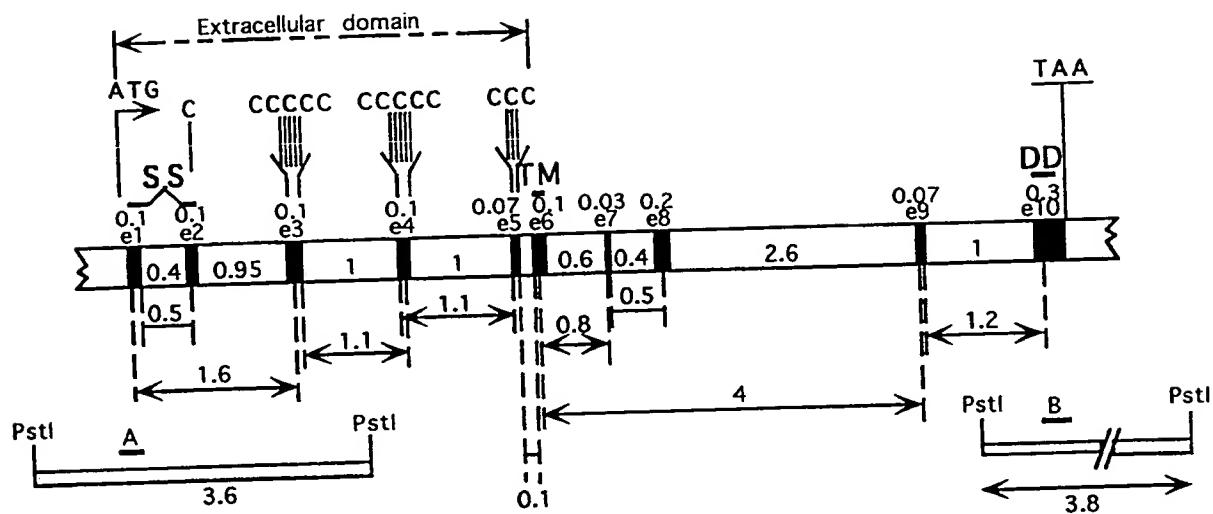


FIG. 8

FIG. 9

10 20 30 40 50 60
TGGCTCGTCTGTTCCCTACGGCCCCCTCCGCATGGCCTCCACCCCAGTCAGAAATT
ACCGAGCAGACAAGGAGATGCCGGGAGGCGTACCGCGAGGTGGGTCAAGTCTTTAA

70 80 90 100 110 120
TCTGCCTCAAGCTCAGGGAGCTGGTACTGAGGTACAGGTAGGAGAAGGTGCAGCCTCTGC
AGACGGAGTTCGAGTCCCTCGACCRTGACTCCATGTCCATCCTCTTCACGTCGGAGACG

130 140 150 160 170 180
TGAGTGGAGGACATGTGCCCTGGGATCCAGAGTCTANAGTACTGACACCAGCCCTGCA
ACTCACCTCCGTACACGGGACCCCTAGGTCTCAGATNTCACTGACTGTGGTCGGAGACG

190 200 210 220 230 240
ATTGTCCCTCTAGACCTGTTAACAGAGCCTGCAGAAGTTACACAGGGCTGCTGAAGAT
TAAACAGGAGGATCTGGACAATTGTCTCGGACGTCTCAAATGTGTCCCGACGACTTCTA

250 260 270 280 290 300
TTTTATCCCTCATTTACAGGAAGGAGCTGGGCCATACCANANTCAGAGCTGGACCGG
AAAATAGGGAGTAAATGTCCCTCTCGACCCGGTAGGTNTNAGTCTCGACCTGGCC

310 320 330 340 350 360
GANCACGGCAGGGCCCANCCATCCACAGCCTCCAATTTCAGTACTCTCTAACAAACCTGAN
CTNGTGGCGTCCCGGGTNGGTAGGTGTCGGAGGTTAAAGTCATGAGAGATTGTGGACTN

370 380 390 400 410 420
ANCCTGANCTTCGGATCTCTTGTATTCTACTTTGATAAAANAAAAGGATGGGTTGGAA
TNGGACTNGGAAGCCTAGAAGAACTAAGGATGAAACTATTNTTTCTACCCAACCTT

430 440 450 460 470 480
AGACTGGCATANAAATTGGACCANTCCGGCATTGACANAACCANGAACTGGGTCGGAA
TCTGACCGTATNTTAACCTGGTNAGGCCGTAACTGTNTGGTNCTGACCCAGGCCTT

490 500 510 520 530 540
CTGAACTCACTCCANGCTCATCTGAATTACACTGACANCCCTGAAGATGGAAAACCTTATT
GACTTGAGTGAGGTNCGAGTAGACTTAAGTGGACTGTNGGACTTCTACCTTTGAATAAA

550 560 570 580 590 600
CCATTAACANGCATNGCGTTGGCANGGACTGGCCCANCTGAACACGGTTTCCAATT
GGTAATTGTNCGTANCGCCAACCGTNCCGTACCGGGGTNGACTTGTGCCAAAGGTTAAT

610 620 630 640 650 660
CACANCTGGGAATTAAANTCTCTCTGCCGCCCTCCCGTGTATTCTTGCCTGGAAATCCCC
GTGTNGACCCCTTAATTNAGAAGAGACGGGGAGGGGACAATAAGAACGGACCTTAGGGG

670 680 690 700 710 720
CCAGTCATCTACTCCCAGTACCAAAATCCTGTATTCTTGAAAATCCGTAGTACTGAGAG
GGTCAGTAGATGAGGGTCATGGTTTAGGACATAAGAACTTTAGGCATCATGACTCTC
10 _____ 20 _____ 8 TO 1109 OF P1-KILLER-1 50 _____ 60 _____ >

730 740 750 760 770 780
GTGACAGCTTGCTGGCAGTCCTCAACAGCCCTCGCTCGCTCTGGCGCCTCTGCC
CACTGTCGAACGACCGTCAGGAGTTGTGGGAGCGAGCGAGAGCCGCGGAGGAGACGGAC
70 _____ 8 TO 1109 OF P1-KILLER-1 110 _____ 120 _____ >

790 800 810 820 830 840
GGCTCCCACTTTGGCGGCACTTGANGAGCCCTCAGCCCACCGCTGCAGTGTGGAGGCC
CGAGGGTGAACCGCCGTGAACTNCTCGGAAGTCGGGTGGCACGTGACACCCCTCGGG

FIG. 9 - cont'd

130 14_8 TO 1109 OF P1-KILLER-1 170 180 >
 850 860 870 880 890 900 900
 TTTTCTGGGCTGGCCAAGGCTGGAGCCCGCTCCCTCAGCTTGACGGAGCTGTGGANGGA
 AAAAGACCCGACCGGTTCCGACCTCGGGCGAGGGAGTCGAACGTGCCACACTNCCT
 190 20_8 TO 1109 OF P1-KILLER-1 230 240 >
 910 920 930 940 950 960
 GAGGCAGCGAGCGGGAACCGGGGCTGCGCGCGGGCACTTGCGGGCCAGCTGGAGTTCCGG
 CTCCCGCGCTGCCCTTGGCCCCGACCGCGCGCCGTGAACGCCCGGTGACCTCAAGGCC
 250 26_8 TO 1109 OF P1-KILLER-1 290 300 >
 970 980 990 1000 1010 1020
 GTGGGCGTGGGCTCGGGGGCCCTGCACTCGGAGCAGCCGGCCGGCCCTGCGCGCCCCGG
 CACCCGCACCGAGCCGCCCCGGACGTGAGCCTCGTGGCCGGCCGGACGGCCGGGGCC
 310 32_8 TO 1109 OF P1-KILLER-1 350 360 >
 1030 1040 1050 1060 1070 1080
 GCAATGANGGTTTAGCACCCGGCCANCGGCTGCGGANGGTGTACTGGGTCCCCCANCA
 CGTTACTNCCCAAATCGTGGGCCGGTNGCCGACGCCNTCCACATGACCCAGGGGTNGT
 370 38_8 TO 1109 OF P1-KILLER-1 410 420 >
 1090 1100 1110 1120 1130 1140
 ATGCCGGCCCACGGCGCTGCGCTTGATTCTCGCCGGCCCTAGCTGCGCTTCCCACGG
 TACGGCCGGGTGACCGCGACCGGAACAAAGAGCGGGCCCGGAATCGACGGAAAGGGCTGCC
 8 TO 1109 OF P1-KILLER-1 >
 1150 1160 1170 1180 1190 1200
 GCAGGGCTGGGACGCCAATGCGCTGAGCCTCTCACCCCTCCGTGGCTCCGTGCAGCC
 CGTCCCAGCCCTGGCGGTACGGACTCGGAGAGTGGGGAGGCACCGAGGACACGTCGG
 1210 1220 1230 1240 1250 1260
 GGAGCCTCCCCGAGGACACCTCCCCCTGCTCCAGGGCGCCAGTCCCACGACCACCA
 CCTCGGAGGGCTGCTGGAGGGGACGAGGTCCCCGGGTCAAGGTAGCTGGTAGGTGGGT
 1270 1280 1290 1300 1310 1320
 AGGGCTGAGGAGTGCAGGGCGCACGGCGGGAGCACGGCGGGACTGGCAGGGAGCTCC
 TCCCACCTCTCACGCCCGCGTGGCGCGCCCTCGTGGCGCGCCCTGACCGTCCGTGAGG
 1330 1340 1350 1360 1370 1380
 ACCTGCAACCCCGGTGCGGGATCCACTGGTGACGACACOTGGCTCCTGAGTCTGGTGG
 TGGACGTTGGGCCACGCCCTAGGTGACCCACTGCTGGACCGAGGACTCAGACCAACC
 1390 1400 1410 1420 1430 1440
 GGACGTGGAGAACCTTATGTTAGCTAAGGGATTGTAATAACACCAATTGGCAGCTGT
 CCTGCACCTCTGGAAATACAATCGATTCCCTAACATTATGTTAGCTGGTAAACCGTGACACA
 1450 1460 1470 1480 1490 1500
 ATCTAGCTCAAGTTTGTAACACACCAATCAGCACCGTGTCTAGCTAGCTAGGGTTGTG
 TAGATCGAGTTCAAAACATTGTGTGGTTAGCTGGCACACAGATCGAGTCCCACAC
 1510 1520 1530 1540 1550 1560
 AATGCACCAATTGACACTCTGTATCTAGCTAGTCTGGTGGGGCCTGGAGAACCTTTGTG
 TTACGTGGTTAATGTGAGACATAGATCGATCAGACCAACCCGGAACCTCTGGAAACAC
 1570 1580 1590 1600 1610 1620
 TCCACACTCTGTATCTAGCTAATCTGGTGGGGAGTGGAGAACATTGTGTCTAGCTCAG

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FIG. 9 - cont'd

AGGTGTGAGACATAGATCGATTAGACCACCCCTCACCTCTGTAAACACAGATCGAGTC
 1630 1640 1650 1660 1670 1680
 GGATTTGTAACACCAATCAGOGCCCTGTCAAACAGACCACTCCGCTCTACCAATCAGC
 CCTAACATTTGGTGGTTAGTCGGGACAGTTTGCTGGTGAGGCAGATGGTAGTCG
 1690 1700 1710 1720 1730 1740
 AGGGTGTGGTGGGGCCANGATAAGAGAATAAAAGCAGGCTGCCAGAGCCAGCAGTGGTA
 TCCCACACCCACCCCGTNCTATTCTCTTATTTCGTCCGACGGTCTGGTCAACCAT
 1750 1760 1770 1780 1790 1800
 ACACTGTGGAAAGTTCCCTCTTCCCTCTGCAATAATCTGCTACTGCTCACTCTTG
 TGTGACACCTCAAAGGAAGAAAGGGAGACGTATTAGAACGATGAACGAGTGAGAAAC
 _____1 TO 2663 OF KILLER(FULL CDNA& & PROMOTER)_0_____>
 _____10____1 TO 815 OF WU/9/12/CHOP_50_____>
 1810 1820 1830 1840 1850 1860
 GGTCCACANTGCTTTATGAGCTTAAACACTCACCTGAAAGGTCTGCAGCTTCACTCTT
 CCAGGTGTACGGAAATACTCGAACATTGTGAGTGGAACTTCCAGACGTCGAAGTGAGAA
 _____60____1 TO 2663 OF KILLER(FULL CDNA& & PROMOTER)_110_____>
 _____60____70____1 TO 815 OF WU/9/12/CHOP_00____110_____>
 1870 1880 1890 1900 1910 1920
 GAGCCAGCGAGACCACGAGGCCACGGGAGGAAAGAACAACTCCAGACCCACTGCCTTA
 CTTCGGTCGCTCTGGTCTCGGGTGGCCCTCTTGTGAGGCTGGGTGACGGAAT
 _____120____1 TO 2663 OF KILLER(FULL CDNA& & PROMOTER)_170_____>
 _____120____130____1 TO 815 OF WU/9/12/CHOP_60____170_____>
 1930 1940 1950 1960 1970 1980
 AGAGCTGTAACACTCACTGGAAAGGTCTGCAGCTTCACTCCTGAGCAGGACACGA
 TCTCGACATTGTGAGTGAACCTTCCAGACGTCGAAGTGAGGACTCGGTCACTCTGGTCT
 _____180____1 TO 2663 OF KILLER(FULL CDNA& & PROMOTER)_230_____>
 _____180____190____1 TO 815 OF WU/9/12/CHOP_20____230_____>
 1990 2000 2010 2020 2030 2040
 ACCCACCGAAGGAAGAAACTCCGAACACATCCGAACATCAGAAGGAACAAACTCCAGAC
 TGGGTGGTCTCCTCTTGAGGCTTGTGAGGCTTGTAGTCTTCTTGTGAGGTCTG
 _____240____1 TO 2663 OF KILLER(FULL CDNA& & PROMOTER)_290_____>
 _____240____250____1 TO 815 OF WU/9/12/CHOP_80____290_____>
 2050 2060 2070 2080 2090 2100
 ACGCCGCCCTTAAGAACTGTAACACTCACCGCGAGGGTCCGAGGCTTCATTCTGAAGGC
 TGCGCGGAAATTCTGACATTGTGAGTGGCGCTCCAGGCTCCGAAGTAAGAACCTCCG
 _____300____1 TO 2663 OF KILLER(FULL CDNA& & PROMOTER)_350_____>
 _____300____310____1 TO 815 OF WU/9/12/CHOP_40____350_____>
 2110 2120 2130 2140 2150 2160
 AGTGAGACCAAGAACCCACCAATTCCGGACACAGTACCATGAAGGAATGAAAATACATAA
 TCACTCTGGTCTGGGTGGTAAAGGCTGTGTCATGGTACTTCTTACTTTATGTATT
 _____360____1 TO 2663 OF KILLER(FULL CDNA& & PROMOTER)_410_____>
 _____360____370____1 TO 815 OF WU/9/12/CHOP_00____410_____>
 2170 2180 2190 2200 2210 2220
 CAATGTGATGTATCATGTTTATTCCTAGACTAGTGACAAATGAAAGCTAAGTGTAGCA
 GTTACACTACATAGTACAATAAGGATCTGATCACTGTTACTTCTGATTACATCGT
 _____420____1 TO 2663 OF KILLER(FULL CDNA& & PROMOTER)_470_____>
 _____420____430____1 TO 815 OF WU/9/12/CHOP_60____470_____>

FIG. 9 - cont'd

2230 2260 2270 2280
 AGGGTGCAGGGACACAGGCACATTGTGQACTAGGTGTGAGTGTAAGCTGGGTTCGATGG
 TCCCACGTCCCTGTGTCGGTAAACACCTGATCCACACTCACATTGACCCAAGCTACC
480 1 TO 2663 OF KILLER(FULL CDNA& & PROMOTER) _530_>
480 490 1 TO 815 OF WU/9/12/CHOP_20_ 530_>

 2290 2300 2310 2320 2330 2340
 TCTTTGGCAACATAGTGAACCCCTGTGTACTAAAAATAACAAAATTAGCCAGGCCT
 AGAAAACCGGGTGTATCAGTGGGACACAGATGATTTATGTTTAATCGGTCCGCA
540 1 TO 2663 OF KILLER(FULL CDNA& & PROMOTER) _590_>
540 550 1 TO 815 OF WU/9/12/CHOP_80_ 590_>

 2350 2360 2370 2380 2390 2400
 GGTGGTGCAGGCCTGTAGTCCCAGCTACATGGGAGGCTGAGGTGGGAGTATCGCTTGAA
 CCACACGTCCGGACATCAGGGTCATGTAACCTCCGACTCCACCCATAGCGAACTTG
600 1 TO 2663 OF KILLER(FULL CDNA& & PROMOTER) _650_>
600 610 1 TO 815 OF WU/9/12/CHOP_40_ 650_>

 2410 2420 2430 2440 2450 2460
 CTGGGAGACGGAAGTTGCACTGAGCCGGATCACACCCAGATCACCAATCTGAGCCACA
 GACCCCTCTGCCCTCAACGTCACCTGGCCCTAGTGTGGGCTAGTGGTTAGACTCGGTGT
660 1 TO 2663 OF KILLER(FULL CDNA& & PROMOTER) _710_>
660 1 TO 815 OF WU/9/12/CHOP_700_>

 2470 2480 2490 2500 2510 2520
 GAGAGACTGTCTAAAAAACCACAGGAAGGGAGGTAGGGGGAGGGGGAGGGGAGGG
 CTCTCTGACAGAGTTTTATTGGTGTCCCTCCCCCATCCCCCTCCCCCTCCCTCCC
720 1 TO 2663 OF KILLER(FULL CDNA& & PROMOTER) _770_>
20 30 1 TO 916 OF RESERVE K-2_60_ 70_>

 2530 2540 2550 2560 2570 2580
 AGGAAAGAGAAAGAGAGAAAGGAAGGAAAGAGAAAGCAGGAAGGACGGAAAGAACGAA
 TCCTTTCTCTTCTCTTCTCTTCTCTCTCTCTCTCTCTCTCTCTCTCTGCTT
780 1 TO 2663 OF KILLER(FULL CDNA& & PROMOTER) _830_>
80 90 1 TO 916 OF RESERVE K-2_120_ 130_>

 2590 2600 2610 2620 2630 2640
 AGAACGAAAGAAAACGAAAGAAAAAGGAAAGAGAAGAGAGAGAGAGAACAGAAGGGCA
 TCTTGCTTTCTCTTCTCTTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTGCTT
840 1 TO 2663 OF KILLER(FULL CDNA& & PROMOTER) _890_>
140 150 1 TO 916 OF RESERVE K-2_180_ 190_>

 2650 2660 2670 2680 2690 2700
 GGTGCCCTGGGAAGGGAGAAGATCAAGACGCGCCTGGAAAGCGGATTNTGAACNTCAA
 CCACGGGGACCTTCCCTCTCTAGTCTCGCGGGACCTTCGCTTAANACTTGAGTT
900 1 TO 2663 OF KILLER(FULL CDNA& & PROMOTER) _950_>
200 210 1 TO 916 OF RESERVE K-2_240_ 250_>

 2710 2720 2730 2740 2750 2760
 GACCCCTGTTACAGCCAAGCGCGGACCCGGGAGGCGTCAACTCCCCAAGTGCCTCCCT
 CTGGGACAAGTGTGGTTCGGCGCTGGGGCCCTCCGCAAGTTGAGGGGTTACGGAGGGA
960 1 TO 2663 OF KILLER(FULL CDNA& & PROMOTER) _010_>
1 TO 916 OF RESERVE K-2_>

 2770 2780 2790 2800 2810 2820

FIG. 9 - cont'd

CAACTCATTCCCCCAAGTTCGGTGCCTGCTGGCGGGACAGGACCCAGAAACAAAC
GTTGAGTAAAGGGGGTCAAAGCACGGACAGGACCGCCCTGCTTGGGTCTTGTTG
1020 1 TO 2663 OF KILLER(FULL CDNA& & PROMOTER) _070_>

2830 2840 2850 2860 2870 2880
CACAGCCCCGGGCAGGCCAGGGCGAAGGTTAGTTCCCTTCCCCTCCCAACTT
GTGTCGGGCCCCGTCGGTCCCGTTCAATCAAGGAAGGGAGGGAGGGTTGAA
1080 1 TO 2663 OF KILLER(FULL CDNA& & PROMOTER) _130_>
->
->

2890 2900 2910 2920 2930 2940
GGAAGGGGTTTGCAGGAGATTGCGTTGACGAGACTCTTATTGTCACCAACCTGTGG
CCTTCCCCAACGCTCCTAACGCAACTGCTCTGAGATAATAACAGTGGTTGGACACC
1140 1 TO 2663 OF KILLER(FULL CDNA& & PROMOTER) _190_>

Oct
2950 2960 2970 2980 2990 3000
TCCAATTGCAATTGATCTGATTGCCCGCCCGAATGACGCTGCCCGAG
ACCTTAACGTCAACGTGTAACCTAGACTAAGCGGGCGGGCTACTGCGACGGGCCTC
1200 1 TO 2663 OF KILLER(FULL CDNA& & PROMOTER) _250_>

3010 3020 3030 3040 3050 3060
GCAGTGAAAGTACAGCCGCCCGCCCAAGTCAGCCTGGACAACATAATCAGCACGCC
CGTCACTTATGTCGGCGGGCGGGGTTAGCTGGACCTGTTGATTAGTCGTGCGCC
1260 1 TO 2663 OF KILLER(FULL CDNA& & PROMOTER) _310_>

3070 3080 3090 3100 3110 3120
CGGGAGAACCCCGCAATCTGCGCCCACAAAAACCCGACGATGCCGATCTACTTTA
GGCCTTGGCGTTAGAGACCGGGGTCTTATGTCGCTACGGGCTAGATGAAAT
1320 1 TO 2663 OF KILLER(FULL CDNA& & PROMOTER) _370_>

3130 3140 3150 3160 3170 3180
putative
trans.
AGGGCTGAAACCCACGGGCCTGAGAGACATATAAGACCGTTCCCTACCGCAGGGAAAC
TCCCAGTTGGTGCCGGACTCTCTGATATCTCGCAAGGGATGGCGGTACCTGTTG
1380 1 TO 2663 OF KILLER(FULL CDNA& & PROMOTER) _430_>
start

3190 3200 3210 3220 3230 3240
GGGGACAGAACGCCCGGCCCTCGGGGCCCGAAAAGGCACGGCCCAGGACCCAGGG
CCCCTGTCTGCGGGCCGGCAAGCCCCCGGGCTTTCCGTGCCGGTCCCTGGGTCCC
1440 1 TO 2663 OF KILLER(FULL CDNA& & PROMOTER) _490_>

3250 3260 3270 3280 3290 3300
AGGCAGGGGAGGCCAGGCCCTGGGCCCCGGTCCCCAAGACCCCTGTGCTCGTTGTGCCG
TCCGGCCCCCTGGTCCGGACCCGGGGCCAGGGGTTCTGGGAAACACGAGAACAGCGGC
1500 1 TO 2663 OF KILLER(FULL CDNA& & PROMOTER) _550_>

3310 3320 3330 3340 3350 3360
CGGTCTGCTGTGGTCTAGCTGAGTCTGCTCTGATCACCAACAAGACCTAGCTCCC
GCCAGGACGACAACCAAGAGTCGACTCAGACGAGACTAGTGGGTTCTGATCGAGGGG
1560 1 TO 2663 OF KILLER(FULL CDNA& & PROMOTER) _610_>

3370 3380 3390 3400 3410 3420
AGCAGAGAGCGGGCCCCACAACAAAAGAGGTCCAGGCCCTCAGAGGGATTGTGTCACCTG
TCGTCCTCGCCGGGTGTGTTCTCCAGGTGGGAGTCCTCCCTAACACAGGTGGAC
1620 1 TO 2663 OF KILLER(FULL CDNA& & PROMOTER) _670_>

3430 3440 3450 3460 3470 3480

FIG. 9 - cont'd

GACACCATATCTCAGAAGACGGTAGAGATTGCATCTCCCTGCAAATATGGACAGGACTATA
 CTGTGGTATAGAGTCTCTGCCATCTCAACGTAGAGGACGTTATACCTGTCTGATAT
 1680_1 TO 2663 OF KILLER(FULL CDNA& & PROMOTER)_730_>
 3490 3500 3510 3520 3530 3540
 GCACTCACTGGAATGACCTCCTTTCTGCTTGCGCCGACCCAGGTGTGATTCAGGTGAAG
 CGTGAGTGAACCTTACTGGAGGAAAAGACGAACGCGGCGTGGTCCACACTAAGTCCACTTC
 1740_1 TO 2663 OF KILLER(FULL CDNA& & PROMOTER)_790_>
 3550 3560 3570 3580 3590 3600
 TGGAGCTAAGTCCCCTGACCCAGCAGAAACACAGTGTGTGAGTGCAGAGAACGGCACCT
 ACCTCGATTCAAGGACGTGGTGTGGCTTGTGTACACAGTCACGCTTCTCCGTGGA
 1800_1 TO 2663 OF KILLER(FULL CDNA& & PROMOTER)_850_>
 3610 3620 3630 3640 3650 3660
 TCCGGGAAGAAGATTCTCCTGAGATGTGCCGGAAGTGCCGCACAGGGTGTCCCAGAGGGA
 AGGCCCTTCTTCTAAAGAGGACTCTACACGGCCTTCACGGCGTGTCCCCACAGGGTCTCCCT
 1860_1 TO 2663 OF KILLER(FULL CDNA& & PROMOTER)_910_>
 3670 3680 3690 3700 3710 3720
 TGGTCAAGGTGGTGTGATTGTACACCCCTGGAGTGACATCGAATGTGTCCACAAAGAACATCAG
 ACCAGTCCAGCCACTAACATGTGGGACCTCACTGTAGCTTACACAGGTGTTCTTAGTC
 1920_1 TO 2663 OF KILLER(FULL CDNA& & PROMOTER)_970_>
 3730 3740 3750 3760 3770 3780
 GCATCATCATAGGAGTCACAGTTGAGCCGTAGTCTGATTGTGGCTGTGTTGTTGCA
 CGTAGTAGTATCCTCAGTGTCAACGTCGGCATCAGAACTAACACCGACACAAACAAACGT
 1980_1 TO 2663 OF KILLER(FULL CDNA& & PROMOTER)_030_>
 3790 3800 3810 3820 3830 3840
 AGTCCTTACTGTGGAAGAAAGTCCTCCTTACCTGAAAGGCATCTGCTCAGGTGGTGGTG
 TCAGGAATGACACCTTCTTCAGGAAGGAATGGACTTCCGTAGACGAGTCACCACAC
 2040_1 TO 2663 OF KILLER(FULL CDNA& & PROMOTER)_090_>
 3850 3860 3870 3880 3890 3900
 GGGACCTCTGGAGCGTGTGGACAGAAGCTCACAAACGACCTGGGGCTGAGGACAATGTCCCTCA
 CCCTGGGACTCGCACACCTGTCTCGAGTGTGCTGGACCCCGACTCCTGTTACAGGAGT
 2100_1 TO 2663 OF KILLER(FULL CDNA& & PROMOTER)_150_>
 3910 3920 3930 3940 3950 3960
 ATGAGATCGTGAGTATCTGAGCCACCCAGGTCCCTGAGCAGGAAATGGAAGTCCAGG
 TACTCTAGCACTCATAGAACGTCGGGTGGTCCAGGGACTCGTCTTTACCTTCAGGTCC
 2160_1 TO 2663 OF KILLER(FULL CDNA& & PROMOTER)_210_>
 3970 3980 3990 4000 4010 4020
 AGCCAGCAGAGCCAACAGGTGTCAACATGTTGCCCCCGGGAGTCAGAGCATCTGCTGG
 TCGGTGCTCGGTTGTCCACAGTTGTACAACAGGGGGCCCTCAGTCTGTAGACGACC
 2220_1 TO 2663 OF KILLER(FULL CDNA& & PROMOTER)_270_>
 4030 4040 4050 4060 4070 4080
 AACCGGCAGAAGCTGAAAGGTCTCAGAGGAGGGCTGCTGGTCCAGCAAATGAAGGTG
 TTGGCCGTCTCGACTTCCAGAGTCTCCTCCTCCGACGACCAAGGTGTTACTTCCAC
 2280_1 TO 2663 OF KILLER(FULL CDNA& & PROMOTER)_330_>
 4090 4100 4110 4120 4130 4140
 ATCCCACGTGAGACTCTGAGACAGTGCTCGATGACTTGCAGACTTGGTGCCTTGTGACT
 TAGGGTGACTCTGAGACTCTGTCACGAAGCTACTGAAACGTCTGAACCACGGAAACTGA

FIG. 9 - cont'd

2340_1 TO 2663 OF KILLER(FULL CDNA& & PROMOTER)_390_>
4150 4160 4170 4180 4190 4200
CCTGGGAGCCGCTCATGAGGAAGTTGGGCCTCATGGACAATGAGATAAAGGTGGCTAAAG
GGACCCCTCGCGAGTACTCCTCAACCCGGAGTACCTGTTACTCTATTCCACCGATTTC
2400_1 TO 2663 OF KILLER(FULL CDNA& & PROMOTER)_450_>
4210 4220 4230 4240 4250 4260
CTGAGGCAGCGGGCCACAGGGACACCTTGATCACGATGCTGATAAAGTGGGTCAACAAAA
GACTCCGTGCCCCGGTGTCCCTGTGGAACATGTGCTACGACTATTCAACCCAGTTGTTT
2460_1 TO 2663 OF KILLER(FULL CDNA& & PROMOTER)_510_>
4270 4280 4290 4300 4310 4320
CCGGGCGAGATGCCCTGTCCACACCCCTGCTGGATGCCCTGGAGACGCTGGGAGAGAGAC
GGCCCGCTCTACGGAGACAGGTGTGGGACGACCTACGGAACCTCTGCGACCCCTCTCTG
2520_1 TO 2663 OF KILLER(FULL CDNA& & PROMOTER)_570_>
4330 4340 4350 4360 4370 4380
TTGCCAACGAGATTGAGGACCACTTGTGAGCTCTGGAAAGTTCATGTATCTAGAAAG
AACGGTTCGTCTCTAACCTCTGGTAACAACTCGAGACCTTCAAGTACATAGATCTTC
2580_1 TO 2663 OF KILLER(FULL CDNA& & PROMOTER)_630_>
4390 4400
GTAATGCAGACTCTGCCATGTCCTAA-
CATTACGTCTGAGACGGTACAGGATT-
26_1 TO 2663 OF KILLER(_>

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/14495

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :C12N 5/00, 15/00, 15/10, 15/12, 15/63

US CL :Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/69.1, 455, 462, 463, 320.1, 325; 536/23.1, 23.5, 24.31; 530/350, 387

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

MEDLINE, BIOSIS, EMBASE, CAPLUS, WPIDS, APS
search terms: death domain, killer, apo 2, tumor necrosis factor receptor, apoptosis, p53, inducible, transgenic, TRAIL

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	ADERKA, D. The Potential Biological and Clinical Significance of the Soluble Tumor Necrosis Factor Receptors. Cytokine & Growth Factor Reviews. 1996, Vol. 7, No. 3, pages 231-240, see entire document.	1-20
A,P	US 5,763,223 A (WILEY et al.) 09 June 1998, see entire document.	1-20
A	WO 97/18323 A3 (ICOS CORPORATION) 22 May 1997, see Abstract.	1-20

 Further documents are listed in the continuation of Box C. See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

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"P" document published prior to the international filing date but later than the priority date claimed

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document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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"A"

document member of the same patent family

Date of the actual completion of the international search
30 SEPTEMBER 1998Date of mailing of the international search report
28 OCT 1998Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231
Facsimile No. (703) 305-3230Authorized officer
JILL D. MARTIN
Telephone No. (703) 308-0196*Open for*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/14495

A. CLASSIFICATION OF SUBJECT MATTER:

US CL :

435/69.1, 455, 462, 463, 320.1, 325; 536/23.1, 23.5, 24.31; 530/350, 387